University of Alberta

The Synthesis and Immunochemistry of S-Linked Gangliosides

by

Jamie Rich

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the

requirements for the degree of *Doctor of Philosophy*

Department of *Chemistry*

Edmonton, Alberta
Fall 2005

Library and Archives Canada
Published Heritage Branch
395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque et Archives Canada
Direction du Patrimoine de l'édition
395, rue Wellington
Ottawa ON K1A 0N4
Canada

0-494-08722-6

Your file *Votre référence*
ISBN:
Our file *Notre reference*
ISBN:

NOTICE:
The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:
L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

Abstract

Vaccines comprised of polysaccharides or oligosaccharides covalently linked to proteins have been shown to stimulate a carbohydrate specific immune response and to prompt development of immunological memory. Accordingly, the creation of vaccines against pathogens for which surface carbohydrates have been identified is an active area of research, and use of fully synthetic oligosaccharide antigens in human vaccine preparations has now been realized. The identification of tumor associated carbohydrate antigens has suggested the possibility that oligosaccharide based vaccines might find use in cancer therapy, though immunogenicity of these preparations remains insufficient.

Modification of oligosaccharide antigens via enzymatic hydrolysis by endogenous glycosidases may hinder the development of a robust immune response through destruction of crucial terminal recognition elements. These include sialyl residues prominent in tumor associated ganglioside glycolipids. The creation of glycosidic linkages resistant to in vivo enzymatic or acid hydrolysis has been proposed to enhance the immunogenicity of glycoconjugates. It remains unknown whether *S*- or *C*-glycosides resemble closely enough the conformation of naturally occurring O-linked oligosaccharides to serve as effective immunogens in this context, and whether in vivo hydrolysis is a significant contributor to their poor immunogenicity.

Described herein are syntheses of analogues of gangliosides GM_3 and GM_2, modified by inclusion of a hydrolysis resistant thiosialosides and a truncated sphingosine aglycon designed to facilitate conjugation to protein or surfaces. Glycosyltransferases of microbial origin catalyzed elaboration of S-linked GM_3 to higher thiooligosaccharide

structures. In addition, for the first time carbohydrate thiols were shown to undergo glycosylation by glycosyltransferases.

S-Linked ganglioside analogues and their O-linked counterparts were coupled with tetanus toxoid and the immunogenicity of these conjugates was evaluated in mice. Two thiooligosaccharides examined induced a strong antigen specific antibody response. Furthermore, IgG and IgM antibodies to the S-linked GM_3 trisaccharide recognized related O-glycosides. Antibodies to either of the synthetic O-gangliosides demonstrated equal or higher affinity towards the related S-glycosides than to the immunizing antigen. This study corroborates earlier work in the Bundle group in which S-linked microbial antigens induced production of antibodies cross reactive with O-glycosides. Recognition of S-glycosides by antibodies, glycosyltransferases and glycosidases indicates a high degree of conformational similarity with the native glycosides, and that thiooligosaccharides may find application in therapeutics.

Acknowledgements

I would like to thank my supervisor, David Bundle for his support and guidance during my time at the University of Alberta. I feel that my decision to join his group was a fortunate one.

This work was made possible only through the assistance of a number of others, both inside and outside the Department of Chemistry. I would especially like to thank Sandra Jacques, with whom I collaborated closely on the chemoenzymatic synthesis of O-linked oligosaccharides. In addition, I would like to single out Joanna Sadowska, who performed all of the ELISA experiments reported in this thesis, and Chang-Chun Ling, who is an invaluable resource to students and others in the Bundle group. Monica Palcic and Warren Wakarchuk provided cloned glycosyltransferases used in this work. I thank Adam Spacenko, Blake Zheng and Lisa Pritchard for expression and purification of these enzymes, and for performing kinetics measurements. Those working in this department will certainly be familiar with the expertise and assistance provided by Angie Morales-Izquierdo (mass spectrometry) and Albin Otter (NMR). I would also like to thank all of the other members of the Bundle group, Vani Ambigakpakan, Soren Andersen, Jamie Bailey, Bernd Becker, Magali Buffet, Ye Cai, Frederic Carrell, Lina Ciu, Stephane Chambert, Darren Derksen, Courtney Doerkson, Sebastian Dziadek, Gordon Grant, Karin Fodor, Jonghwa Kim, Pavel Kitov, Birgit Kranke, Lynne Lechelt, Tomasz Lipinski, Lesley Liu, Scott McGavin, Mark Nitz, Corwin Nycholat, Eugenia Paszkiewicz, Thomas Peters, Hongmei Shang, Dmitry Solomon, Dean Williams, Xiangyang Wu, Henry Yu, Ping Zhang for their assistance and friendship over the years.

Outside of the lab, I want to thank my family for their patience and understanding while I focused on my studies. I am fortunate to have met a number of wonderful people from around the world during my time in Edmonton who I am keen to remain in touch with. Finally, I want to thank Sarah for everything, especially commuting from Australia to be with me.

Table of Contents

		Page
Chapter 1	**Introduction: Thiooligosaccharide Containing Conjugate Vaccines for Cancer Immunotherapy**	**1**
1.1	Carbohydrates in biological systems	1
1.2	Carbohydrate based conjugate vaccines	2
1.3	Carbohydrate based cancer immunotherapy	8
1.3.1	Tumor associated carbohydrate antigens	8
1.3.2	Gangliosides in cancer immunotherapy	10
1.3.3	Augmenting the immunogenicity of ganglioside anti-cancer vaccines	12
1.4	Thiooligosaccharides	14
1.4.1	General features	14
1.4.2	Synthesis of thiooligosaccharides	16
1.4.3	Application of thiooligosaccharides	20
1.5	Scope of the project	21
Chapter 2	**The Synthesis of Protected *S*-Linked Sialosides for Incorporation into Conjugate Vaccine Preparations**	**23**
2.1	Introduction	23
2.2	The synthesis of thiosialosides for use in conjugate vaccines	26
2.2.1	Targets	26
2.2.2	Synthesis of *S*-linked analogues of Neu5Acα(2→6')lactose	27
2.2.3	Synthesis of α(2→3) linked thiosialyl galactosides and lactosides	31
2.2.4	Attempted synthesis of 8-thio Neu5Ac	47

2.3	Conclusions	49
		Page
Chapter 3	**Chemical and Chemoenzymatic Synthesis of *O*- and *S*-Linked Ganglioside Analogues and Fragments Thereof for the Synthesis of Glycoprotein Conjugates**	**50**
3.1	Introduction	50
3.2	Chemical and enzymatic strategies for the synthesis of gangliosides	52
3.3	Target molecules	55
3.4	Synthesis of *S*-linked glycosphingolipid analogues from protected thiosialosides	56
3.4.1	Elaboration of α(2→6') thiosialyl lactose	56
3.4.2	Synthesis of *S*-linked GM_3 from α(2→3) thiosialyl galactose and α(2→3') thiosialyl lactose	58
3.4.3	Synthesis of *O*-linked ganglioside analogues	65
3.5	Thioglycosylceramides	69
3.6	Conclusions	71
Chapter 4	**Glycosyltransferase Catalysed Thiooligosaccharide Synthesis**	**72**
4.1	Introduction	72
4.2	Attempted glycosyltransferase catalysed glycosylation of carbohydrate thiols	74
4.2.1	Transfer to 3'-thiolactose	74
4.2.2	Synthesis and evaluation of 4'-thiolactosides as glycosyltransferase substrates	80
4.2.3	Synthesis of potential acceptors for the human blood group glycosyltransferases A and B	83
4.3	Conclusions	86

		Page
Chapter 5	**Immunochemistry of *S*-Linked Oligosaccharides**	**87**
5.1	Introduction	87
5.2	Characteristics of the antibody response	88
5.3	Immunization	90
5.4	Synthesis of protein conjugates	93
5.5	Immunization of experimental animals	97
5.5.1	Analysis of antibody levels in immune sera	97
5.5.2	Comparison of the antibody response to *O*- and *S*-glycosides	106
5.6	*S*-Glycosides as immunogens: speculation and conclusions	109
Chapter 6	**Conclusions and Future Directions**	**113**
Chapter 7	**Experimental Section**	**117**
	References	**244**

List of Figures

Figure	Title	Page
1.1	B-cell mediated immune response to carbohydrate antigens.	6
1.2	Some tumor associated carbohydrate antigens.	10
1.3	Some naturally occurring thiosugars.	15
1.4	General features of glycosidic bond hydrolysis.	16
1.5	General methods for the synthesis of thioglycosides.	16
1.6	General features of (a) retaining glycosidases (b) glycosynthases, and (c) thioglycoligases.	19
2.1	The basic structures of the neuraminic acids.	23
2.2	The most commonly occurring sialosyl linkages.	24
2.3	Target *S*-linked analogues of GM_3 and GM_2 oligosaccharides.	27
2.4	Facile elimination in gulopyranosides.	47
3.1	Truncated azidosphingosine (2*S*,3*R*) 2-azido-3-O-benzoyl-pent-4-ene-1,3-diol provides flexibility in coupling to proteins.	52
3.2	Interactions (dis)favouring glycosidation of sphingosine and azidosphingosine.	53
3.3	Target oligosaccharides for use in glycoprotein conjugates.	55
5.1	Vaccine and screening glycoconjugates prepared from (a) GM_3 and (b) GM_2 analogues.	96
5.2	Miscellaneous screening glycoconjugates.	97
5.3	Binding of IgG from polyclonal sera derived by immunization with *S*-GM_3-TT glycoconjugate **124** to *S*-GM_3-BSA glycoconjugates **126** and **127**.	100

Figure	Title	Page
5.4	Binding of IgG from polyclonal sera derived by immunization with *S*-GM_2-TT glycoconjugate **129** to *S*-GM_2-BSA glycoconjugates **131** and **132**.	100
5.5	Binding of IgG and IgM from polyclonal sera derived by immunization with conjugate thiotrisaccharide **124** to thiotetrasaccharide **132**, and from sera raised against the thiotetrasaccharide **129** to thiotrisaccharide **127**.	101
5.6	Binding of IgG from polyclonal sera derived by immunization with *S*-GM_3 conjugate **124** to *S*-GM_3 **127** and *O*-GM_3 **128**.	102
5.7	Binding of IgG from sera against *S*-GM_3 glycoconjugate **124** to BSA glycoconjugates **134-136**.	103
5.8	Synthetic oligosaccharides **87** and **88** inhibit binding of IgG from polyclonal sera against *S*-GM_3-TT conjugate **124** to immobilized *O*-GM_3-BSA **128**.	104
5.9	Glycosides used in inhibition experiments.	105
5.10	Inhibition of antibody binding to *O*-GM_3-BSA conjugate **128** by *S*- and *O*-linked oligosaccharides **137**, **52,** and **89**.	106
5.11	Binding to immobilized *O*-GM_3-BSA **128** and *S*-GM_3-BSA **127** by murine IgG generated from immunization of mice with *O*-GM_3-TT **125** or *S*-GM_3-TT **124**.	107
5.12	Binding to immobilized *S*-GM_2-BSA **132** and *O*-GM_2-BSA **133** by murine IgG generated from immunization with *S*-GM_2-TT **129** or *O*-GM_2-TT **130**.	108
5.13	Titration of monoclonal IgM antibody GMR-6 against *S*-GM_3-BSA (**127**) and *O*-GM_3-BSA (**128**).	109
5.14	Thiooligosaccharide analogues of microbial antigens used as immunogens.	112

List of Schemes

Scheme	Title	Page
2.1	(a) Glycosylation with neighbouring group participation. (b) Glycosylation and elimination processes in reactions of Neu5Ac.	25
2.2	Methods for the synthesis of α(2→6′) linked thiosialosides.	28
2.3	Retrosynthetic analysis of protected α(2→6′) thiosialyl lactose.	29
2.4	Attempted synthesis of α(2→6′) thiosialyl lactose by displacement of the 6′-bromide.	30
2.5	Synthesis of a protected α(2→6′) thiosialyl lactoside.	31
2.6	Reported syntheses of *S*-linked Neu5Acα(2→3)Gal.	32
2.7	Attempted epimerization of a benzyl protected galactoside to a guloside.	34
2.8	Synthesis of a protected 3-thiogalactoside.	35
2.9	Synthesis of 3-thiogalactoside **29** from D-gulono-γ-lactone.	36
2.10	Synthesis of protected α(2→3) thiosialyl galactose.	38
2.11	Epimerization at C-3′ of an acetylated lactose derivative.	40
2.12	Potential precursors to protected 3′-thiolactose.	41
2.13	Proposed route for formation of **33** and **36** via acyloxonium ion intermediates.	42
2.14	Synthesis of 3′-thiolactose peracetate.	42
2.15	Preparation of protected 4-thio-glucose and 4-thio-galactose.	43
2.16	Synthesis of protected 3′-thiolactoside **44**.	44
2.17	Synthesis and partial deprotection of α(2→3′) thiosialyl lactose.	46
2.18	Potential route to α(2→8) thiodisialosides.	48
2.19	Regioselective *O*→*N* acyl migration from *O*-8 to *N*-5 of Neu5Ac.	49

Scheme	Title	Page
3.1	Synthesis and deprotection of an *S*-linked ganglioside analogue containing an α(2→6′) thiosialosyl linkage.	57
3.2	Retrosynthesis of a protected thio-GM_3 intermediate.	59
3.3	Synthesis of protected glucosyl ceramide homologues.	60
3.4	Proposed mechanism for formation of **67** by intermolecular acyl transfer.	61
3.5	Synthesis and deprotection of *S*-linked GM_3 analogue **52**.	62
3.6	Enzymatic elaboration of *S*-GM_3 to sulfur containing analogues of GM_2 (**54**) and GD_3 (**78**).	64
3.7	Synthesis and deprotection of *S*-linked glycolipids.	65
3.8	Synthesis of acceptor substrates for α(2,3) sialyltransferase.	67
3.9	Enzymatic synthesis of GM_3 and GM_2 oligosaccharides.	68
3.10	Chemical and enzymatic synthesis of sialyl galactosides.	69
3.11	Synthesis of a 1-thio-azidosphingosine homologue.	70
4.1	Idealized biocatalytic thiooligosaccharide synthesis using thiol modified donors/acceptors.	73
4.2	Synthesis of a 3'-thiolactoside as a potential glycosyltransferase substrate.	76
4.3	Synthesis of hemiacetal **102** via hydrolysis of the glycosyl bromide.	76
4.4	Sequential transfer of α-linked galactosyl residues to thiol **100**.	77
4.5	(a) Attempted sialylation of thiol **100** and (b) glycosylation by α(1,4) galactosyltransferase.	78
4.6	Glycosylation of thiol **100** by β(1,3) *N*-acetylglucosaminyltransferase.	80
4.7	Preparation of 4'-thiolactoside **110**.	81

Scheme	Title	Page
4.8	Synthesis of protected 4'-thiolactoside **114**.	82
4.9	Synthesis of a 3-*S*-acetyl protected H-disaccharide analogue.	84
4.10	Synthesis of a 3-*S*-benzyl protected H-disaccharide analogue.	85
4.11	Attempted glycosylation with a triethylsilyl protected fucose donor.	86
5.1	Synthesis of glycoconjugates using adipate and squarate linkers.	95
5.2	ELISA protocols for determination of titre and IC_{50} values.	98

Abbreviations

Ac	acetyl
AcOH	acetic acid
AcSH	thiolacetic acid
ADCC	antibody dependent cell mediated cytotoxicity
APC	antigen presenting cell
All	allyl
$BF_3{\cdot}Et_2O$	boron trifluoride diethyl etherate
Bn	benzyl
BnBr	benzyl bromide
Boc	*t*-butyloxycarbonyl
Boc_2O	di-*tert*-butyl dicarbonate
BSA	bovine serum albumin
Bz	benzoyl
BzCl	benzoyl chloride
t-Bu	*t*-butyl
CAN	ceric ammonium nitrate
CMC	complement mediated cytotoxicity
CMP-Neu5Ac	cytidine-5'-monophospho-*N*-acetylneuraminic acid
CSA	camphorsulphonic acid
CTL	cytotoxic T-lymphocyte
DBU	1,8-diazabicyclo-[5.4.0]-undec-7-ene
DCM	dichloromethane
DEAD	diethyl azodicarboxylate
Determinant	see epitope
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DTT	D/L-dithiothreitol
ELISA	enzyme linked immunosorbent assay
ESI HRMS	electrospray ionization high resolution mass spectrometry

Et	ethyl
Et_3Si	triethylsilyl
Fab	antigen binding fragment
Gal	D-galactose
GalNAc	*N*-acetylgalactosamine, 2-acetamido-2-deoxy-D-galactose
GalT	galactosyltransferase
GCOSY	gradient coupling correlated spectroscopy
Glc	glucose
GlcNAc	*N*-acetylglucosamine, 2-acetamido-2-deoxy-D-glucose
GlcNAcT	*N*-acetylglucosaminyl transferase
GSL	glycosphingolipid
GTA	blood group glycosyltransferase A
GTB	blood group glycosyltransferase B
HMBC	heteronuclear multiple bond correlation
HMQC	heteronuclear multiple quantum coherence
HPLC	high performance liquid chromatography
HRP	horseradish peroxidase
IC_{50}	inhibitor concentration required to give 50% inhibition
Ig	immunoglobulin
KLH	keyhole limpet hemocyanin
$KOBu^t$	potassium *tert*-butoxide
KSAc	potassium thioacetate
MALDI-TOF	matrix assisted laser desorption ionization time of flight
Me	methyl
MeCN	acetonitrile
MHC	major histocompatibility complex
MS	molecular sieves
Ms	methanesulfonyl
Neu	neuraminic acid; 3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid

NeuAc	*N*-acetylneuraminic acid; 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid
NeuGc	*N*-glycolylneuraminic acid; 3,5-dideoxy-5-*N*-glycolyl-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid
NMR	nuclear magnetic resonance
OGr	8-methoxycarbonyloctyl
OSE	2-(trimethylsilyl)ethyl
OTf	trifluoromethanesulfonate
PBS	phosphate buffered saline
PBST	phosphate buffer saline containing Tween 20
Ph	phenyl
PMB	4-methoxybenzyl
PPh_3	triphenylphosphine
ppm	parts per million
pyr.	pyridine
RT	room temperature
SepPak	solid phase extraction cartridge
SiaT	sialyltransferase
S_N2	bimolecular nucleophilic substitution
$SnCl_4$	tin tetrachloride
TACA	tumor associated carbohydrate antigen
TCR	T-cell receptor
TFA	trifluoroacetic acid
Tf_2O	trifluoromethanesulfonic anhydride
T_{H2}	T-helper lymphocyte type 2
THF	tetrahydrofuran
TLC	thin layer chromatography
TMSOTf	trimethylsilyl trifluoromethanesulfonate
Tr	trityl (triphenylmethyl)
TT	tetanus toxoid
p-TSA	*p*-toluenesulfonic acid

TSCA	tumor specific carbohydrate antigen
UDP-Gal	uridine 5'-diphosphogalactose
UDP-GalNAc	uridine 5'-diphospho-*N*-acetylgalactosamine
UDP-GlcNAc	uridine 5'-diphospho-*N*-acetylglucosamine
UV	ultraviolet
Z	benzyloxycarbonyl

Chapter One

Introduction: Thiooligosaccharide Containing Conjugate Vaccines for Cancer Immunotherapy

1.1 Carbohydrates in biological systems

Biomacromolecules are normally classified as nucleic acids, protein, carbohydrate or lipid in nature. The three former groups are differentiated from lipids by their polymeric composition, and carbohydrates are further distinguished from other biological polymers in two important respects: they are frequently non-linear, and even identical monosaccharide subunits may be combined to form a multitude of positional and stereochemical isomers. Carbohydrate chemistry and biology have suffered as a consequence of the inherent complexity of these molecules. Until relatively recently carbohydrates were appreciated for little more than their roles in energy storage, transport and in some cases as skeletal components. Similarly, prior to the 1970's the synthesis of even common disaccharides, when attainable, was achieved only in specialist laboratories.

Given that all cells display a dense array of glycoconjugates or polysaccharides at their surface and that cells must interact with other cells and surrounding biomolecules to function, it is not surprising that carbohydrates are involved in important molecular recognition events. Saccharides have been shown to mediate in-*cis* and in-*trans* cellular recognition events, including processes such as inflammation, immune response, fertilization, embryonic development, host-pathogen interactions, and dynamic regulation of cytoplasmic protein function.[1-9] Glycobiology, a term first advanced by Dwek in

1988,[10] is a rapidly expanding, broad field of research that seeks to elucidate and comprehend the roles of oligo- and poly-saccharides in a biological context.

Insofar as glycoconjugates such as glycoproteins, glycolipids, and glycosaminoglycans are media for the relay of biological information, the information is translated via numerous highly specific low affinity carbohydrate-carbohydrate or carbohydrate-protein (immunoglobulin, lectin, or enzyme) binding events.[11,12] Growing understanding of these phenomena in recent years has led to a revision of the traditional view of cellular recognition events in which homotypic protein-protein interactions were paramount.[13] Multivalent binding, the simultaneous association of two or more receptors and their ligands, provides an avenue by which carbohydrate binding events can attain the binding strength and specificity required for the mediation of biological information transfer.[14]

Oligosaccharides are involved in a variety of molecular recognition events that present opportunities for therapeutic intervention in cancer, tuberculosis, HIV, influenza and other important diseases.[15-17] Glycotherapeutics have received a great deal of attention recently, and undoubtedly glycobiology research will continue to unveil opportunities for their development. In general, realized or proposed therapies employ one or more of the following strategies: inhibition of carbohydrate processing enzymes that are up-regulated or present only in the diseased state, interference with carbohydrate protein or carbohydrate-carbohydrate interactions, immunization with carbohydrate antigens, or the exploitation of carbohydrate-protein binding specificity in drug delivery.[18] Continued investigation of the molecular recognition processes in which

carbohydrates are active will provide insight into the design of new molecules and strategies for interference with and study of fundamental biological processes.

1.2 Carbohydrate based conjugate vaccines

The dense layer of carbohydrates found on the outer surface of the cell membrane renders these molecules attractive antigens as they are substantially more accessible to the immune system than lipids, which are largely concealed within the cell membrane, and proteins, which are often masked by carbohydrate residues. An additional advantage is that as secondary gene products, carbohydrates sequences are somewhat more conserved than protein antigens.

The success of active immunotherapy is dependent on (i) the ability of the immune system to recognize an antigen as foreign, (ii) the ability to mount a response to the intended target resulting in its clearance, and (iii) the establishment of acquired immunity (immunological memory). Despite their availability to the immune system and their conserved structural features, carbohydrates are typically poor immunogens.

The low immunogenicity of poly- and oligosaccharides is a consequence of their incompatibility with common antigen presenting pathways.[19] The immune response is mediated by a series of molecular recognition events, the success of which determines the ultimate immunogenicity of the antigen. The innate immune response involves a number of processes by which molecules are identified as foreign and are cleared from the body, but does not provoke a memory response. Polysaccharides are of sufficient size to cross link surface immunoglobulin (Ig) on B-lymphocytes, prompting the production and secretion of low affinity pentameric antibodies of the IgM subtype (primary humoral response), which are well suited to activate the complement pathway

(innate immunity) and thereby effect clearance. Many bacterial polysaccharides are also recognized by pattern recognition receptors on the surface of macrophages. These recognition events stimulate the secretion of effector molecules that in turn activate innate response pathways. The innate and primary humoral immune responses to capsular polysaccharides on bacteria are often sufficient to provoke their removal, but these responses are T-cell independent and thus do not generate immunological memory. Oligosaccharides are normally of insufficient size to initiate cross linking or bind to pattern recognition receptors and are therefore poorly immunogenic.

The first step in acquired immunity involves recognition of a foreign molecule by surface Ig. Subsequent endocytosis and proteolytic degradation of the antigen-Ig complex provides small fragments which, if bound by major histocompatibility complex (MHC) class II molecules, are transported to the cell surface for presentation to the immune system. Simultaneous recognition of the MHC bound peptide fragment and portions of the MHC molecule by a T-cell receptor (TCR) on the surface of a $CD4^+$ T-helper lymphocyte (T_{H2}), in conjunction with specific secondary intercellular recognition events, results in proliferation of the antigen specific B-cell. These lymphocytes undergo affinity maturation and isotype switching to produce carbohydrate specific IgG in place of IgM (secondary humoral response). During clonal expansion Ig producing B-cell genes undergo repeated somatic mutation and ultimately B-cells that produce IgG of higher affinity are selected. This lymphocyte population is divided into circulating B-cells that secrete IgG (plasma cells) or which are rapidly activated upon re-encountering the antigen (memory cells).

Carbohydrates alone fail to prompt a secondary humoral immune response owing to the specificity of the MHC II molecule for short peptide sequences. However, one or more copies of a polysaccharide or multiple oligosaccharide epitopes appropriately coupled to a carrier protein have been shown to induce antibody production and stimulate immunological memory. The large polysaccharide or multiple oligosaccharides allow for recognition and cross linking of the carbohydrate by B-lymphocyte surface Ig which promotes internalization and degradation. Peptide fragments from the degraded glycoprotein are bound by the MHC II molecule, allowing the successful recruitment of T-cell help that results in the production of carbohydrate specific IgG and establishment of memory B-cells. This process also results in the establishment of an immune response toward fragments of the carrier protein.

Adaptive immunity mediated by a cytotoxic T-lymphocyte response is distinct from the humoral response. Naïve T-cells circulate in the blood and lymph, and are capable of distinguishing between foreign or self MHC-peptide complexes. Upon interaction between a naïve T-cell and an antigen presenting cell (APC) bearing a foreign peptide, the T-cell is activated and its progeny become cytotoxic T-cells that can directly recognize and destroy other cells bearing this peptide. The TCR demonstrates specificity for both the MHC molecule and the foreign molecule to which it is bound. In this way, self peptides presented by MHC do not stimulate an autoimmune response. Although there is evidence that certain glycopeptides and zwitterionic polysaccharides may be presented by MHC proteins, a large glycan is thought to block recognition of peptide residues by the TCR and thus development of a carbohydrate specific CTL response to an oligosaccharide would be unusual.[20]

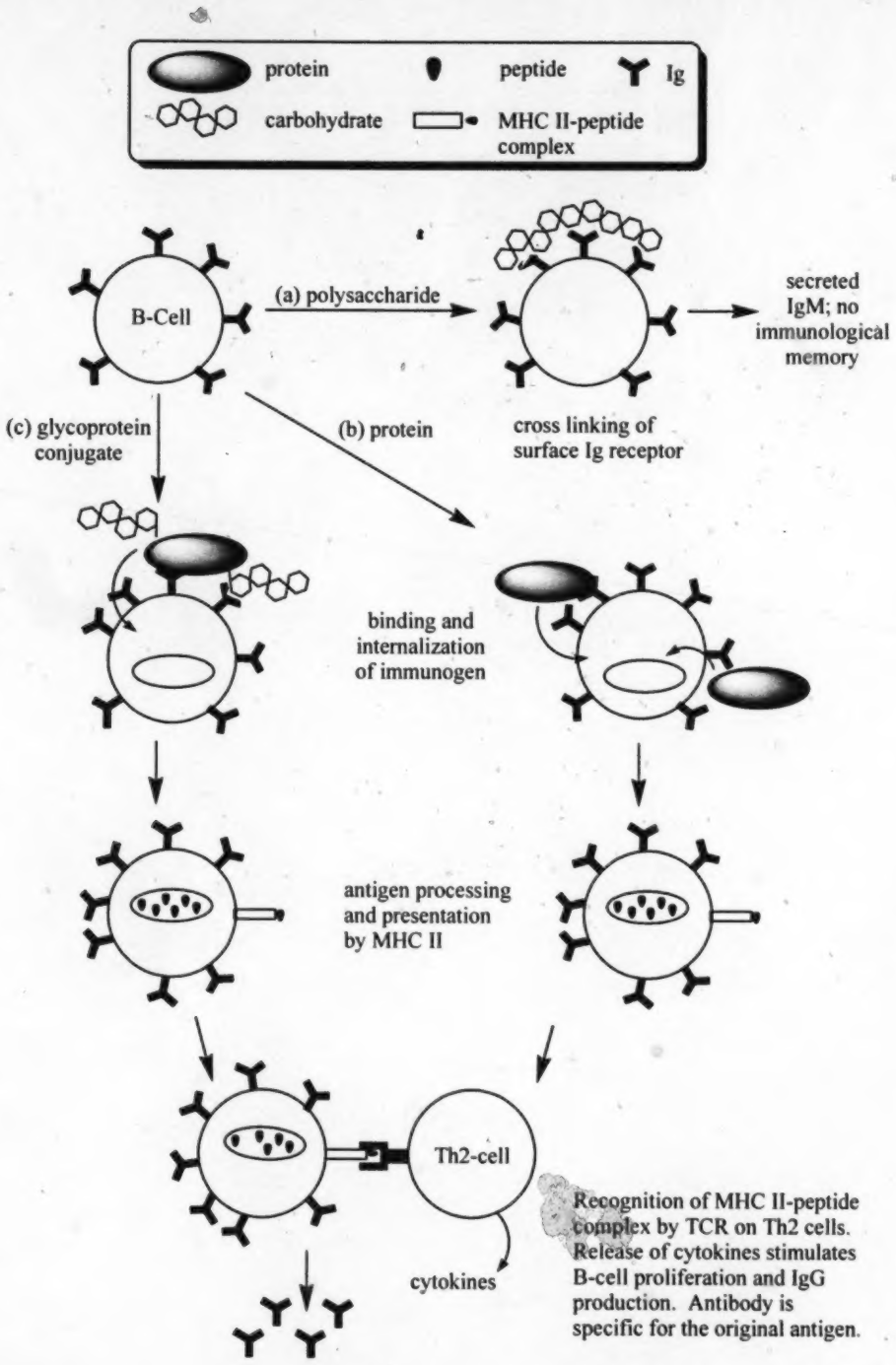

protein
peptide
Ig
carbohydrate
MHC II-peptide complex
B-Cell
(a) polysaccharide
secreted IgM; no immunological memory
(c) glycoprotein conjugate
(b) protein
cross linking of surface Ig receptor
binding and internalization of immunogen
antigen processing and presentation by MHC II
Th2-cell
cytokines
Recognition of MHC II-peptide complex by TCR on Th2 cells. Release of cytokines stimulates B-cell proliferation and IgG production. Antibody is specific for the original antigen.

Figure 1.1. *B-cell mediated immune response to carbohydrate antigens.*

The CD1 family of antigen presenting molecules, found on dendritic and other antigen presenting cells, is related in structure to the MHC I proteins, and has been shown to present glycolipids to natural killer T-cells in a manner analogous to MHC-peptide presentation.[21] The CD1 antigen presentation pathway is an area of intense research that may eventually provide an alternative means of generating an induced carbohydrate-specific immune response. It is important to keep in mind that details of carbohydrate antigen processing remain poorly understood. For example, it has been shown that both CD1 antigen presenting molecules and a subset of MHC I associated T-cells are required for an antibody response to what are traditionally considered T-cell independent polysaccharide antigens.[22]

Despite the current limitations of carbohydrate immunogens, they have great potential as immunotherapeutics. Antibodies are considered the primary mechanism by which pathogens are eliminated, and are ideally suited to clear circulating cells.[23] Upon recognition of the tumor cell by antibody, a number of effector mechanisms of the immune system may result in destruction of this target. An antibody-dependent cell-mediated cytotoxic (ADCC) response involves recognition of the antibody F_C region by phagocytic or natural killer cells, which subsequently internalize and destroy the complex. The complement pathway can also invoke cell death via complement mediated cytotoxicity (CMC) after recognition of the antigen-antibody complex via the F_C portion of the Ig. Antibodies have also been shown to induce apoptosis through alternate mechanisms. Numerous carbohydrate based vaccines are used clinically to combat infectious agents such as *Neisseria meningitides* and *Streptococcus pneumoniae*.[24,25]

The first fully synthetic carbohydrate conjugate vaccine, directed against *Haemophilus influenzae* type b has recently been licensed.[26]

1.3 Carbohydrate based cancer immunotherapy

1.3.1 Tumor associated carbohydrate antigens

Cells undergo dramatic morphological changes during oncogenesis. These include defined alterations to the nature and distribution of cell surface molecules that distinguish malignant from normal cells. Mechanisms by which tumors avoid activation of the immune system have been identified, but an understanding of these processes is in its infancy. Identification of tumor cells as abnormal by the immune system would provide a means of oncotherapy.

The cell surface undergoes quantitative and qualitative changes in glycosylation during malignant transformation.[27,28] While cancer cells may occasionally express a total of 5×10^5 protein antigens at their surface, the average number of carbohydrate molecules is frequently much higher, with expression of up to 5×10^7 molecules of a single antigen documented.[29] Glycan structures that are associated with normal tissues but which are found in higher concentration on tumor cells are known as tumor associated carbohydrate antigens (TACAs). A lesser number of tumor specific carbohydrate antigens (TSCAs) – molecules not expressed on healthy cells, expressed in immunologically privileged tissues, or expressed only during development – have been identified. The immune system is capable of discriminating between benign and malignant cells based on the expression of TSCAs or TACAs and consequently these molecules are potential antigens for therapeutic cancer vaccine development.

Cancer associated carbohydrate antigens have been identified by immunohistochemical staining using monoclonal antibodies (mAbs) of known specificity, lectin binding assays, and by isolation of glycolipid antigens by extraction and thin layer chromatography (TLC). Two distinct groups of glycoconjugates comprise TACAs: carbohydrates bound to the sphingolipid ceramide associated with the lipid bilayer (glycosphingolipids or GSLs) and carbohydrate constituents of cell-surface glycoproteins (Figure 1.2).

The gangliosides are a family of over thirty acidic GSLs that contain at least one sialic acid residue, most commonly *N*-acetylneuraminic acid, linked via an $\alpha(2\rightarrow3)$ or an $\alpha(2\rightarrow6)$ glycosyl linkage to galactose or *N*-acetylgalactosamine units, or via an $\alpha(2\rightarrow8)$ linkage to another sialic acid residue. These molecules are normal components of the outer surface of virtually all mammalian cell membranes. Gangliosides are classified according to the nature of their carbohydrate component. The widely accepted Svennerholm nomenclature refers to the number of sialic acid residues (M = mono, D =di, T = tri, Q = tetra) and describes the relative mobility of the glycolipid within that series on a silica gel TLC stationary phase.[30] Ganglioside GM_3 contains a single sialic acid residue, which is the third most polar among monosialogangliosides. Many gangliosides are over expressed in tumors, rendering them TACAs and potential targets for cancer immunotherapy. In addition, the ganglioside content of malignant cells has been correlated with metastatic potential.[31] GM_3, GM_2, GD_3, and GD_2 are associated with many melanomas and are the most extensively investigated GSL TACAs to date.

1.3.2 Gangliosides in cancer immunotherapy

The identification and characterization of tumor associated antigens has highlighted the exciting prospect of employing the patient's own immune system in an

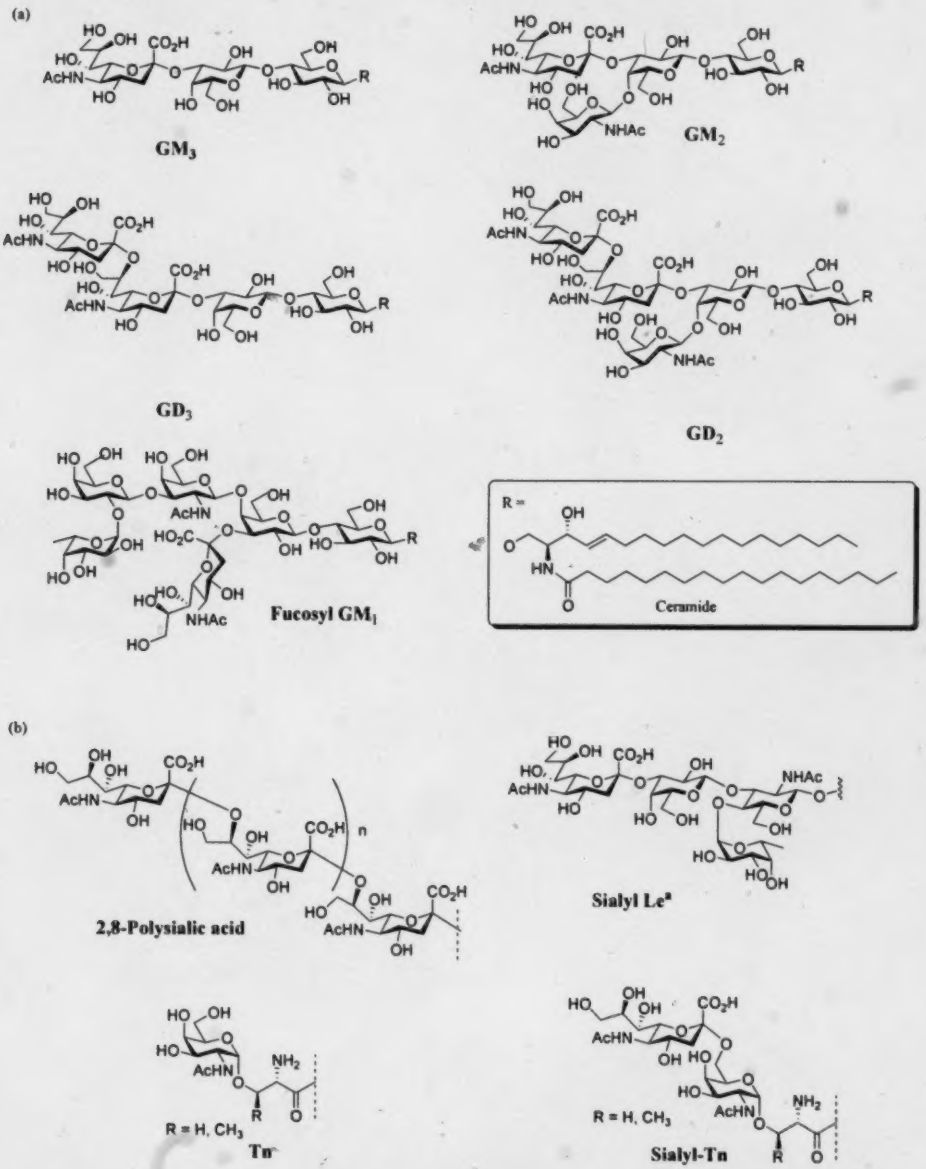


Figure 1.2. *Some tumor associated carbohydrate antigens. (a) Common melanoma associated gangliosides. (b) Some other glycopeptide and glycolipid TACAs.*

effort to control cancer. Each class of antigen, and indeed each individual antigen, has associated advantages and disadvantages from practical, financial, immunological and other standpoints. Ganglioside glycolipids have received attention due to the accessibility of the carbohydrate to the immune system, the high concentration of antigen on malignant cells, their widespread expression in tumors from different patients, and their relatively high degree of structural conservation in the face of the extreme genetic instability associated with carcinogenesis. Disadvantages of ganglioside based immunotherapy include the T-cell independent nature of the unmodified immunogen, the inability of even carbohydrate-protein conjugate vaccines to induce a CTL response, the immune system's tolerance of endogenous molecules, and the potential for autoimmune response if this tolerance is broken. Despite these drawbacks, gangliosides remain promising immunotherapeutic targets.

Initial attempts to establish an immune response to gangliosides and other GSLs relied on immunization with autologous or allogenic tumor cells and their lysates.[32] Clinical efforts to stimulate immunological memory led to the use of purified gangliosides, which proved non-immunogenic, followed by gangliosides in combination with killed bacteria, which yielded IgM responses in some patients.[33,34] Covalent and non-covalent attachment of gangliosides to immunogenic carrier proteins has been demonstrated to generate moderate IgG responses in a number of clinical trials.[35,36]

It is now well established that patients with certain cancers who have natural or induced antibodies against TACAs, including gangliosides, have a more favourable prognosis than patients without the antibodies.[37,38] Although it has been demonstrated that synthetic vaccine constructs are capable of breaking immune tolerance to

ganglioside tumor antigens with minimal autoimmune response,[39] the immunogenicity of these preparations must be enhanced significantly if they are to be clinically effective.

1.3.3 Augmenting the immunogenicity of ganglioside anti-cancer vaccines

A variety of tactics have been advocated to minimize immunotolerance to TACAs in vaccine constructs. The most successful approaches have involved the selection of appropriate immunogenic carrier proteins, and co-administration of highly immunostimulatory adjuvant preparations.[40] The carbohydrate content of conjugate vaccines and the linker chemistry employed have also proven relevant to the immune response.[41,42] An alternative approach, which can be employed in conjunction with a protein carrier and adjuvant, involves subtle modification of the antigen. Alterations can be envisaged that render the antigen more immunogenic by reducing its self character, that enhance its *in vivo* stability and therefore increase its availability to the immune system, or that employ some combination of these strategies.

The successful modification of an antigen in this context has rather paradoxical requirements: the molecule must be physically altered in such a way that it is viewed as foreign by the immune system and/or is not affected by normal pathways for chemical or enzymatic degradation, while at the same time its conformation must remain as similar as possible to the native structure. Thus, with an increase in the intensity of the immune response, there is the potential for decrease in the fidelity of native antigen-antibody recognition. The relative degree to which these phenomena occur should dictate the success of the approach.

Functional group modification has been demonstrated to effectively reduce the self character of some carbohydrate antigens. For example, vaccines containing GD_3

lactone are more immunogenic in mice and humans than unmodified GD_3.[43] Likewise, replacement of the *N*-acetyl group of *N*-acetylneuraminic acid containing oligosaccharides with other acyl groups may enhance the antibody response to conjugate vaccines.[44,45] However, a strong immune response does not necessarily imply improved recognition of the unmodified antigen.[46] Vaccines in which the immunogen is a three-dimensional mimic of the target antigen, but has non-carbohydrate molecular composition, have shown promise.[47,48]

The stability of an immunogen to chemical or enzymatic degradation is important in synthetic vaccine design. Peptide based vaccines are subject to *in vivo* degradation by proteases. A number of strategies have been advanced to enhance their stability, including cyclization, alteration of the amide bond, end capping with D-amino acids, and incorporation of non-proteinogenic amino acids.[49-52] Replacement of the interglycosidic oxygen atom or ring oxygen atom of oligosaccharides with sulfur, carbon or nitrogen typically results in their resistance to hydrolysis by both chemical and enzymatic processes, and has been proposed as a means to enhance the immune response.[53-56] Several of these analogues have been studied *in vitro*,[54,55] but there are no reports evaluating the antibody response to *S*-, *C*-, or *N*-glycosides *in vivo*.

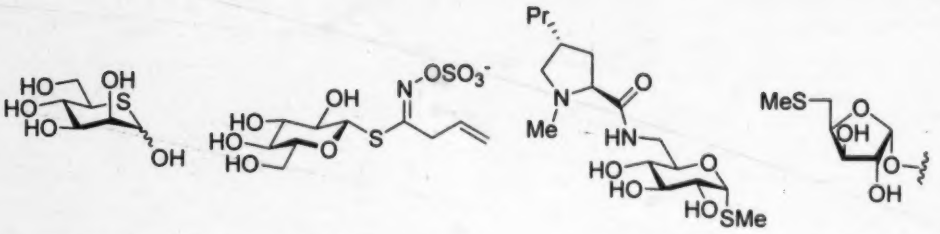
A complementary strategy for enhancing the immunogenicity of carbohydrate antigens involves presentation of a self antigen in the unique context of the target antigen, for example at the abnormally high density observed on the tumor cell surface. The conjugation of multivalent "clusters" of a ganglioside epitope or epitopes to protein carriers may result in antibodies with "context specificity",[57] capable of differentiating between normal and malignant tissues.

It has been demonstrated *in vitro* that human plasma membrane sialidase specific for gangliosides can degrade glycolipid components of neogangliproteins and adjacent cells.[58-60] The action of this glycosidase on conjugate vaccines would destroy crucial terminal recognition elements of tumor associated gangliosides. In addition to preserving the integrity of the antigen, the combined use of hydrolysis resistant gangliosides and epitope clustering could minimize tolerance of TACAs and result in an Ig response with enhanced specificity for tumor cells. *S*-linked glycosides display suitable stability, adopt similar conformations to *O*-glycosides, and are available by chemical synthesis.

1.4 Thiooligosaccharides

1.4.1 General features

A wide range of carbohydrate analogues containing sulfur have been synthesized, while a much lesser number of naturally occurring carbohydrates with this modification have been identified. Saccharides in which an oxygen atom has been replaced by bivalent sulfur are termed thiosugars and can be further classified according to the nature and position of the atomic substitution. Thioglycosides bear a sulfur atom in place of the anomeric oxygen atom, whereas molecules in which the ring oxygen has been replaced by sulfur are termed thiasugars. Thiooligosaccharides are di- to oligosaccharide analogues in which at least one of the interglycosidic oxygen atoms has been replaced by sulfur. Some naturally occurring thiosugars are shown (Figure 1.3). No naturally occurring thiooligosaccharides are known.



5-Thio-D-mannopyranose | Siningrin (a glucosinolate) | Lincomycin | 5-methylthio-α-D-xylofuranoside

Figure 1.3. *Some naturally occurring thiosugars.*

Replacement of the anomeric oxygen atom with sulfur has a variety of conformational implications. The *C-S* bond (1.8Å) is considerably longer than the *C-O* bond (1.4Å), and the *C-S-C* bond angle is considerably smaller (99°) than that formed by the *C-O-C* bonds (114°). The combined effects of these changes result in an increase (0.35 Å) in the distance between the two carbon atoms participating in the glycosidic linkage. In addition to the new geometrical constraints imposed on the thioglycoside, the overall conformation is influenced by modified stereoelectronic and steric contributions. *S*-Glycosides are in general more flexible about the glycosidic linkage than are *O*-glycosides, owing to a diminished *exo*-anomeric effect and a reduction in the magnitude of inter-residue steric interactions.[61] A survey of some free and protein bound thiooligosaccharide conformations reveals that while these analogues adopt very similar conformations to *O*-glycosides, they also populate conformations precluded by their higher energy in the *O*-linked series.[62]

Thioglycosides demonstrate considerably enhanced resistance toward both chemical and enzymatic hydrolysis. It is generally accepted that in both cases hydrolysis of *O*-glycosides proceeds via an oxonium ion as identified in Figure 1.4. The stability of thioglycosides has been rationalized in terms of the reduced solution phase basicity of the

sulfur atom relative to oxygen, and more recently, in terms of the enhanced stability of the sulfonium ion relative to the oxonium species in an enzyme active site.[63,64] *S*-Glycosidases are known, primarily in the plant kingdom, which hydrolyze simple thioglucosides and thioglycosyl pyrimidines.[65] Polysaccharide lyases, enzymes responsible for glycosaminoglycan degradation, have also been shown to hydrolyze thioglycosides.[66] Available structural data suggests that some hydrolases acting on thioglycosides do so without acid catalysis.[67-69]

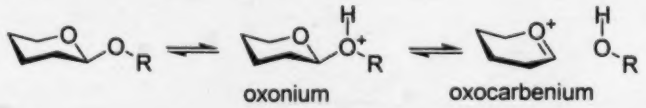


Figure 1.4. *General features of glycosidic bond hydrolysis.*

1.4.2 Synthesis of thiooligosaccharides

Thiooligosaccharides have traditionally been synthesized via one of three strategies: (i) S_N2 displacement of a leaving group on a saccharide by a glycosyl thiolate, (ii) S_N2 displacement of an anomeric leaving group by a sugar thiolate, or (iii) by glycosylation of a carbohydrate thiol with a suitable glycosyl donor (Figure 1.5).[70-72] A variety of alternative approaches have been applied including Michael addition of thiols to α,β unsaturated carbonyl containing carbohydrate analogues,[73] epoxide and aziridine ring opening with thiolates,[74] and chemoenzymatic methods.[75,76]

As synthetically useful thiosugars are not found in nature, sulfur must be introduced into carbohydrates by chemical synthesis. In the majority of situations, this is accomplished via S_N2 displacement of a leaving group by a hydrogen sulfide equivalent at the desired location on the saccharide backbone.[71,72,77] Common nucleophiles include thioacetate and thiocyanate; leaving groups include halides, sulfonate esters, and cyclic

sulfates. With the exception of reactions at the anomeric centre, displacements at secondary carbon atoms normally proceed well only with activated sulfonate leaving groups such as trifluoromethanesulfonates. Mitsonobu conditions, employing thioacetic acid as nucleophile, are frequently used to convert primary alcohols to thioesters. In addition to displacement of an anomeric halide, acid catalyzed reaction of peracetylated sugars with thiols, Fischer type glycosidation, thioacetolysis of 1,6-anhydro sugars, glycosidation of thioacetic acid with glycosyl fluoride or glycosyl trichloroacetimidate donors and radical addition to glycals are other methods used to install sulfur at the anomeric position that take advantage of the unique reactivity of this centre.[74,78-82]

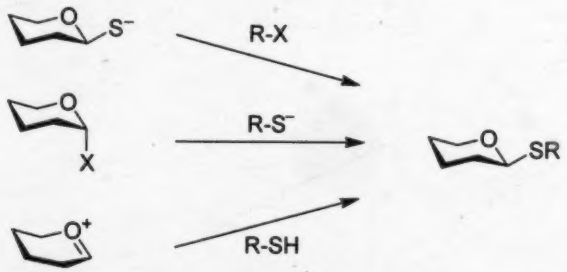


Figure 1.5. *General methods for the synthesis of thioglycosides.*

The identification of an appropriate method for coupling saccharides via a thioglycosidic bond is very much dependent on the nature of the two carbohydrate components and their cost and availability. The formation of stereoisomeric products under standard glycosylation conditions ("acid catalyzed thioglycosidation") constitutes a serious disadvantage to this approach, and as a consequence it is more likely to find use only when the substrates are base sensitive or displacement reactions fail. Stereoisomeric mixtures are occasionally observed when the anomeric thiolate is employed as a nucleophile ("anomeric *S*-alkylation"). It is typically easier to perform

displacement reactions at the anomeric center than it is at unactivated 2° carbon atoms on the saccharide backbone. As a result, it is frequently more efficient to first install the sulfur atom on the residue nearer the reducing terminus for eventual reaction with a glycosyl halide ("base promoted *S*-glycosidation").

As an alternative to chemical synthesis, Withers' group has introduced mutant glycosidases termed glycosynthases[83] for oligosaccharide synthesis, and have recently extended this methodology to achieve chemoenzymatic synthesis of thiooligosaccharides.[75,76] Glycosidases catalyze hydrolysis with either inversion or retention of anomeric configuration. It is in some cases possible to shift the equilibrium towards reverse hydrolysis (glycosidic bond formation) by lowering the water activity or adding an excess of aglycon.[84] Glycosidases which catalyze *O*-glycosyl bond formation have not been shown to utilize thiols as acceptors, with the exception of simple aliphatic molecules.[85,86]

A generic mechanism now broadly accepted for many retaining enzymes is outlined in Figure 1.6(a).[87] Hydrolysis proceeds via a double displacement mechanism in which one carboxyl residue serves as a catalytic nucleophile, establishing a glycosyl-enzyme intermediate, and the other carboxyl residue serves as a general acid-base catalyst. Glycosynthases, on the other hand, are missing the general acid-base residue. As a consequence a glycosyl fluoride does not react with the enzyme to generate a covalent intermediate as shown in Figure 1.6(b). Instead, the nucleophilic residue serves as a base, enhancing the nucleophilicity of the second bound carbohydrate residue and promoting displacement of the fluorine atom. Importantly, the enzyme is unable to hydrolyze the product. Thioglycoligases are also mutant retaining glycosidases that lack

the general acid-base residue, but glycosidic bond formation is effected by a slightly different strategy (Figure 1.6(c)). Dinitrophenyl glycosides are accommodated by the enzyme active site and do not require acid catalysis to form a glycosyl-enzyme intermediate. The strong nucleophilicity of a synthetic carbohydrate-thiol subsequently permits formation of the thioglycoside bond without base catalysis. A variant of this strategy involves "thioglycosynthases" mutants in which both catalytic residues are ablated.[76] *O*-Glycosynthases do not seem to catalyze thioglycoside formation.[88]

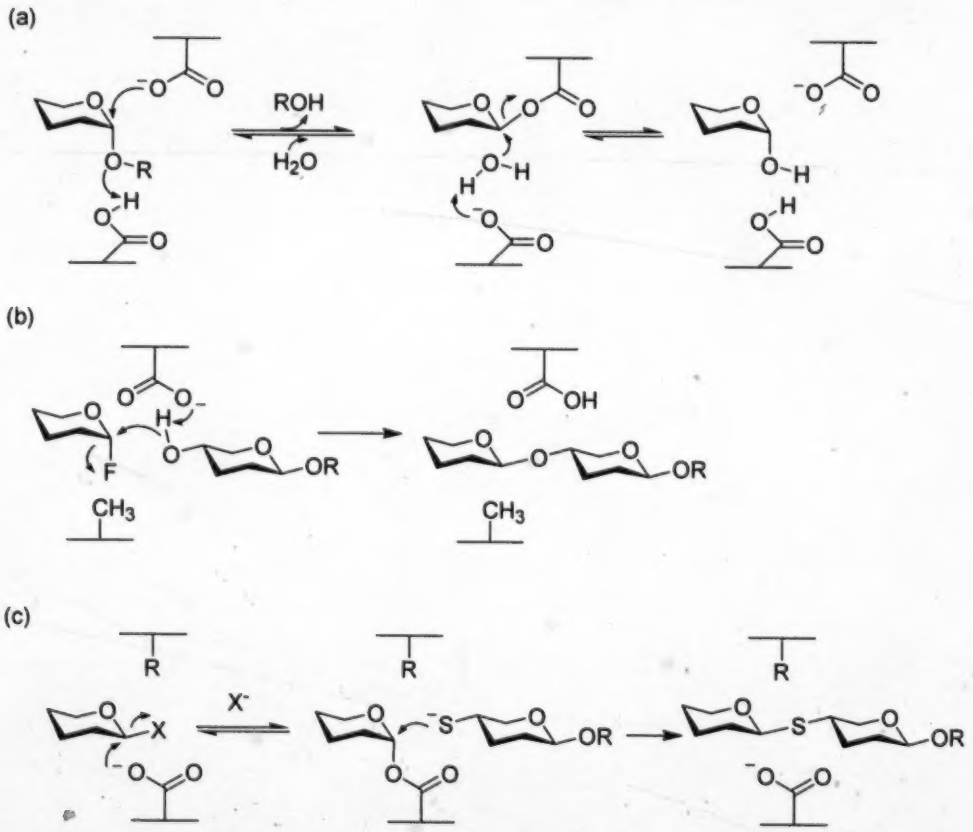


Figure 1.6. *General features of (a) retaining glycosidases (b) glycosynthases, and (c) thioglycoligases.*

The challenges in chemical thiooligosaccharide synthesis are primarily encountered during S_N2 reactions at secondary carbon atoms. Elevated temperatures and

polar aprotic solvents are frequently required to accomplish displacement at secondary centres. As a consequence, competing reactions including elimination, ring contraction and neighbouring group participation may be difficult to suppress. The generation of disulfide dimers during thioglycosidic bond formation can be problematic, although they are often recyclable. By virtue of the presence of sulfur, the use of certain reagents used in protecting group manipulations is restricted, including electron transfer reagents, oxidants, desulfurizing agents, tin reagents and palladium catalysts used in hydrogenolysis. Likewise, use of the many thiophilic promoters used in glycosylation chemistry may be limited. While these reactions are not necessarily prohibited, it is a dangerous to assume that they will proceed readily.

1.4.3 Applications of thiooligosaccharides

Thioglycosides have found application and offer promise in a number of fields including biotechnology, medicine and structural biology.[89,90] *S*-Linked oligosaccharides are widely used as substrate analogues in the study of enzyme structure, function and mechanism.[72] As these heteroanalogues often bind at the active site of glycoside hydrolases with similar affinity to *O*-glycosides but are not subsequently degraded, otherwise unavailable crystallographic complexes are formed.[91-97] Owing to their conformational similarity, thiooligosaccharides, like simple *S*-glycosides, may also serve as competitive inhibitors of glycosidases.[98-102] Application of this class of molecules to protein binding studies is not limited to enzymes. They are employed as non-degradable lectin ligands,[90] may be used on affinity supports for the purification of proteins,[103] and have been used to induce enzymatic activity.[104] The ability to

construct glycoproteins bearing glycans with one or more hydrolytically stable thioglycosidic linkage is an attractive prospect and is an active area of research.

1.5 Scope of project

The following chapters describe the synthesis of ganglioside analogues in which the terminal sialic acid residue is attached via a thioglycosidic linkage, the synthesis of their *O*-linked counterparts, conjugation of the oligosaccharides to protein, and a comparison of the immunogenicity of these two classes of oligosaccharides in mice. During the course of this work we were able to evaluate the capacity of a range of proteins that have evolved to interact with native carbohydrates for their ability to bind to thiooligosaccharides and carbohydrate thiols.

The bulk of the synthesis described involves the construction of thiosialyl oligosaccharides on a truncated sphingosine analogue. The interconversion of gulosides to 3-thiogalactosides proved challenging and was achieved in good yield only following optimization and development of an understanding of the influence of protecting groups on these reactions. After acquiring a number of *S*-linked sialyl galactose building blocks, these structures were elaborated via chemical and enzymatic steps to provide oligosaccharides for coupling to proteins. During the course of this work the ability of glycosyltransferases to catalyze thiooligosaccharide synthesis was explored in detail.

Some of the work included in this thesis has been published or submitted for publication:

(a) Rich, J.R., Szpacenko, A., Palcic, M.P., Bundle, D.R. (2004) *Glycosyltransferase Catalyzed Synthesis of Thiooligosaccharides*. Angew. Chem. Int. Ed. 43: 613-615.

(b) Rich, J.R., Bundle, D.R. (2004) *S-Linked Ganglioside Analogues for Use in Conjugate Vaccines*. Org. Lett. 6: 897-900.

(c) Rich, J.R., Wakarchuk, W.W., Bundle, D.R. (2005) *Chemical and Chemoenzymatic Synthesis of S-Linked Ganglioside Analogues and Their Protein Conjugates for Use as Immunogens.* Chem. Eur. J. Accepted.

(d) Bundle, D.R., Rich, J.R., Jacques, S.J., Yu, H.N., Nitz, M. (2005). *Thio-oligosaccharide Conjugate Vaccines Evoke Antibodies Specific for Native Antigens.* Angew. Chem. Int. Ed. Accepted.

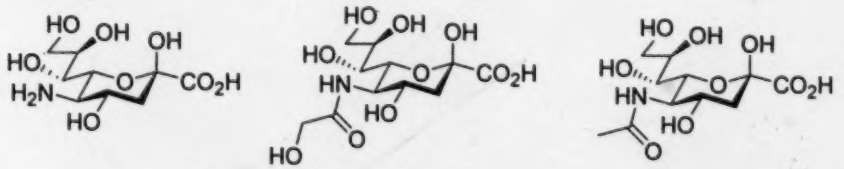
(e) Jacques, S., Rich, J.R., Bundle, D.R. (2005) *Chemoenzymatic Synthesis of GM_3 and GM_2 Gangliosides Containing a Truncated Sphingosine Functionalized for Glycoconjugate and Solid Phase Applications.* Manuscript in preparation.

Chapter Two

The Synthesis of Protected *S*-Linked Sialosides for Incorporation into Conjugate Vaccine Preparations

2.1 Introduction

The sialic acids are a family of over thirty derivatives of the nine carbon ketose neuraminic acid (5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid) that are generally *N*-acylated to form either *N*-acetyl (Neu5Ac) or *N*-glycolyl (Neu5Gc) neuraminic acid (Figure 2.1). Owing to its predominance, *N*-acetyl neuraminic acid is frequently referred to simply as sialic acid. Equilibrium mixtures of the reducing sugars are comprised almost exclusively of the β-anomer, but naturally occurring glycosides sialic acids exist only in the α-configuration with the exception of the β-linked sugar nucleotide donor cytidine monophosphate sialic acid. The conformations of sialic acids are heavily biased towards the $^2C_5$ chair conformer.



neuraminic acid (Neu) | *N*-glycolylneuraminic acid (Neu5Gc) | *N*-acetylneuraminic acid (Neu5Ac)

Figure 2.1. *The basic structures of the neuraminic acids. The β-anomers are shown.*

Sialic acids normally occur as glycosides in glycolipids, glycoproteins and oligosaccharides, and exist almost exclusively as terminal sugars linked to galactose [α(2→3) or α(2→6)], or *N*-acetyl galactosamine [α(2→6)], except when attached to another sialic acid residue via an α(2→8) or α(2→9) linkage. The most common

naturally occurring glycosidic linkages involving Neu5Ac are depicted in Figure 2.2. Sialic acids have been implicated in a range of biological processes, primarily involving their dual and seemingly opposite effects in molecular and cellular recognition. It has been demonstrated that these molecules serve to mask important subterminal oligosaccharide epitopes, and are also themselves critical recognition elements for a variety of receptors and pathogens.[105]

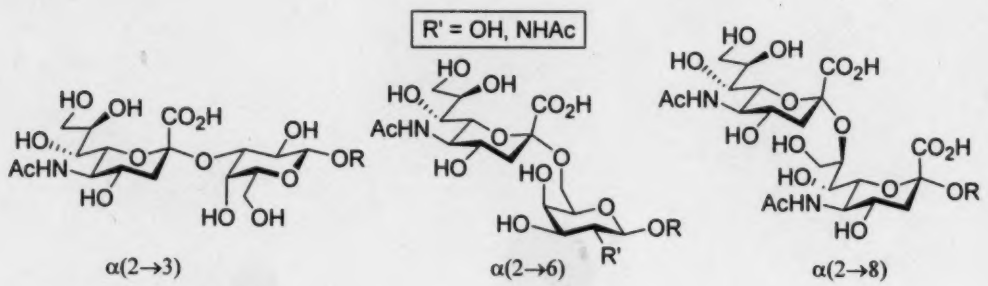
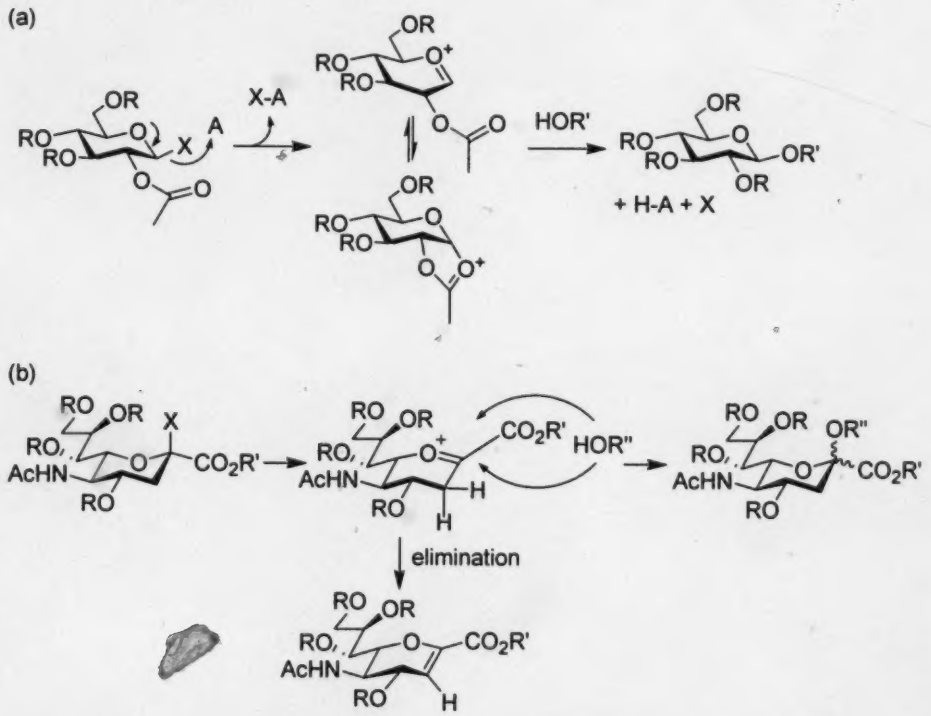


Figure 2.2. *The most commonly occurring sialosyl linkages.*

The chemical synthesis of *O*-linked sialosides is particularly demanding owing to the presence of a quaternary anomeric centre and 3-deoxy-ulosonic acid motif. Glycosylation reactions typically proceed via generation of an oxocarbenium ion followed closely by substitution with an alcohol (Scheme 2.1(a)).[106,107] A participating group such as an ester at the position adjacent to the glycosyl centre allows for stabilization of the requisite oxocarbenium ion and biases the stereochemical outcome of the substitution reaction. The oxocarbenium generated during sialoside formation (Scheme 2.1(b)) is destabilized by the electron withdrawing nature of the C-1 carbonyl group, favouring elimination of H-3 and resulting in the formation of the glycal. Stereoelectronic factors bias the stereochemical outcome towards the undesired β-sialoside, the formation of which engenders an antiperiplanar orientation of the C_1-O_1

bond and a lone pair on the endocyclic oxygen atom that enables an $n \rightarrow \sigma^*$ stabilizing interaction. In addition, the absence in the sialic acids of a hydroxyl group at C-3 prevents utilization of a neighbouring group participation strategy, and contributes to the generation of anomeric mixtures.



Scheme 2.1. *(a) Glycosylation with neighbouring group participation. (b) Glycosylation and elimination processes in reactions of Neu5Ac.*

Owing to the enhanced nucleophilicity and reduced basicity of sulfur relative to oxygen, many thioglycosides can be prepared by nucleophilic displacement of an appropriate leaving group, normally chloride, from the anomeric centre. Apart from some glycal that is often produced in the process, this route generally results in the

formation of a single product stereoisomer in contrast to standard glycosidation techniques.

2.2 The synthesis of thiosialosides for inclusion in conjugate vaccines

2.2.1 Targets

To evaluate the ability of *S*-linked analogues of TACAs to generate antibodies that may recognize *O*-linked gangliosides, access to both *O*- and *S*-linked TACAs and their fragments were required. The terminal sialosyl residues of linear tumor associated gangliosides such as GM_3 and GD_3 (Fig. 1.2) are known to be susceptible to chemical and enzymatic hydrolysis. The TACAs GM_3 and GM_2 were identified as the structurally most simple gangliosides with which to evaluate the potential of *S*-linked immunogens, and hence their sialosyl residues were selected for modification. Although GM_2 is not generally prone to enzymatic hydrolysis outside the lysosomal environment,[108-110] it seemed prudent to examine the impact of vicinal glycosylation on the conformation of a thiosialoside in an immunological context. To obtain the target oligosaccharides **1** and **2** (Figure 2.3) and potential access to other Neu5Ac containing TACAs, methods for the construction of Neu5Acα(2→3[S])Gal and Neu5Acα(2→3[S])Galβ(1→4)Glc fragments were required. A distinction is drawn between these two fragments, despite their obvious homology, owing to the ready availability of the disaccharide lactose and to the importance of distinct di- and tri-saccharide epitopes in numerous TACAs. A Neu5Acα(2→3[S])Gal building block would afford considerable flexibility in the synthesis of a wide array of TACA analogues, while the construction of Neu5Acα(2→3[S])Galβ(1→4)Glc directly from lactose and sialic acid would offer the most direct route to thiogangliosides such as the primary targets **1** and **2**. Methods for

the construction of Neu5Acα(2→6[S])Gal and Neu5Acα(2→8[S])Neu5Ac disaccharides, key components of thio-linked analogues of other complex TACAs, were also investigated.

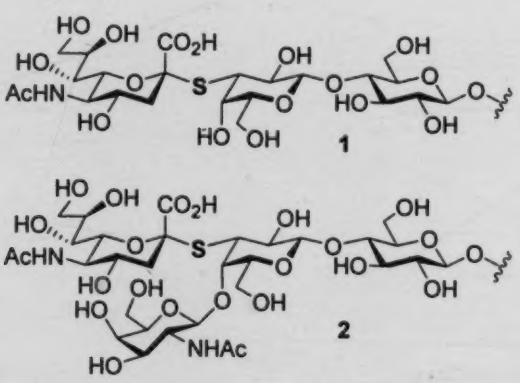
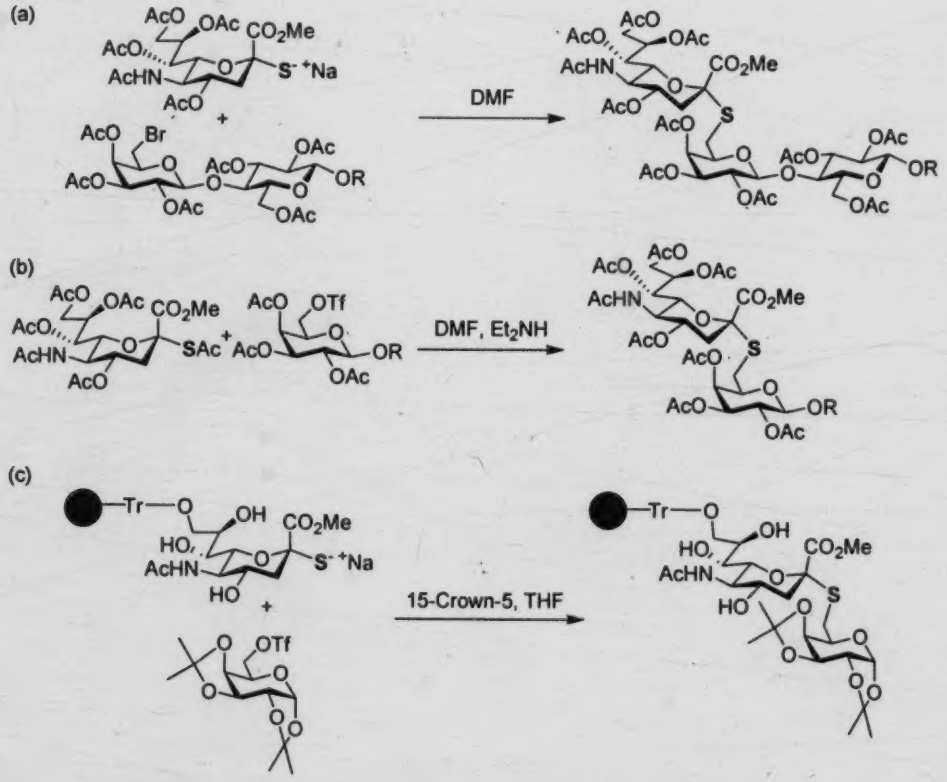


Figure 2.3. *Target S-linked analogues of GM_3 and GM_2 oligosaccharides.*

2.2.2 Synthesis of *S*-linked analogues of Neu5Acα(2→6′) lactose

The α(2→6) sialyl galactose disaccharide is a constituent of hepatoma associated antigens.[111] Owing to the relative simplicity of installing sulfur in place of a 1° alcohol, even in galactosides,[112,113] the synthesis of α(2→6′) thiosialyl lactose trisaccharide served as an appropriate starting point. Neu5Acα(2→6[S])Gal containing oligosaccharides have been synthesized previously,[114,115] and were shown to bind and inhibit influenza virus sialidase.[102] Hasegawa employed the thiolate derived from reaction of the anomeric thioacetate with sodium methoxide at low temperature,[116] followed by reaction with a 1° bromide (Scheme 2.2(a)).[115] A secondary reprotection step was required owing to partial deacetylation. von Itzstein substantially improved on this method, employing a secondary amine to effect *in situ* de-*S*-acetylation followed by reaction with the triflate (Scheme 2.2(b)).[117,118] Finally, Hindsgaul reported a solid

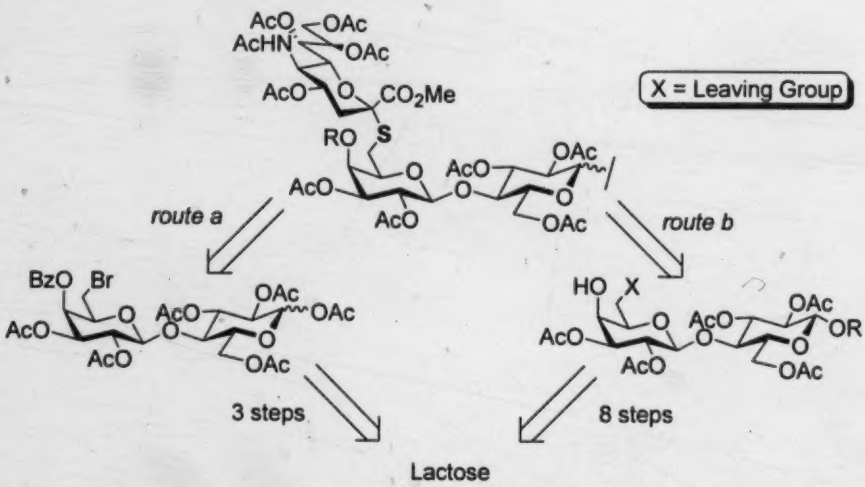
phase method for the synthesis of thiooligosaccharides, which employed unprotected sugar thiolates immobilized on a polystyrene resin (Scheme 2.2(c)).[119] Blocking of the galactosyl residue with base stable protecting groups avoided formation of a product mixture due to deacetylation. A common feature of these reactions is the use of ester and acetal protecting groups to avoid potential incompatibility with the sulfide during deprotection.



Scheme 2.2. *Methods for the synthesis of α(2→6′) linked thiosialosides.*

Two general approaches to the synthesis of α(2→6′) thiosialyl lactose, a regioisomer of the target thio-GM_3 oligosaccharide, are shown in Scheme 2.3. The absence of a discrete glycosidic protecting group on the lactoside (*route a*) would allow

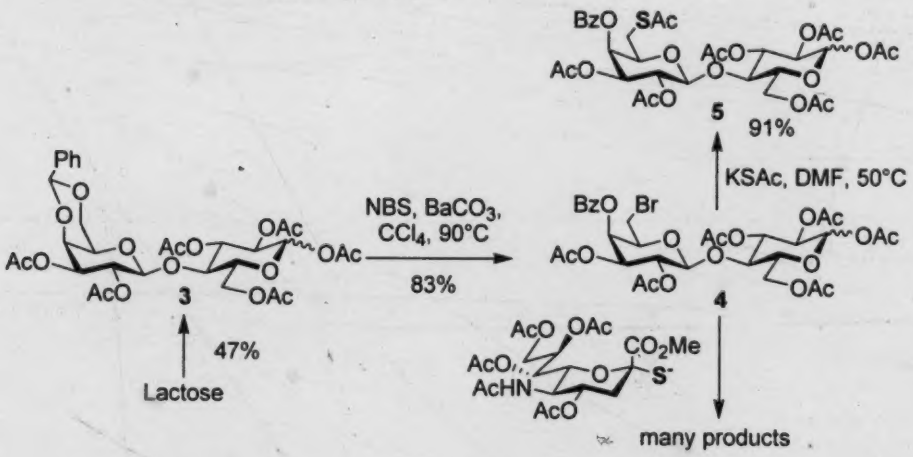
for preparation of the bromide in only three steps from lactose, and would allow for direct conversion of the thiotrisaccharide into an anomeric bromide or trichloroacetimidate for subsequent elaboration to the glycosphingolipid target. By contrast, the use of a glycoside as a protecting group (*route b*) would lengthen the synthesis considerably, but would impart greater stability to the anomeric centre. In either case, the use of acyl protecting groups would allow for a straightforward one-pot transesterification-saponification deprotection sequence.



Scheme 2.3. *Retrosynthetic analysis of protected α(2→6′) thiosialyl lactose.*

Preparation and reaction of the 6′-bromolactose derivative was initially favoured, owing to the relative ease of its preparation (Scheme 2.4). The acetylated 4′,6′-acetal **3** was synthesized by benzylidenation of lactose,[120] followed by acetylation in acetic anhydride-pyridine. Subsequent oxidative cleavage of the benzylidene acetal under Hannessian-Hullar conditions afforded the 4′-*O*-benzoyl 6′-bromide **4**.[121] Unfortunately, reaction with the Neu5Ac derived thiolate under either the conditions reported by Hasegawa[115] or von Itzstein[117] failed to afford substantial amounts of the

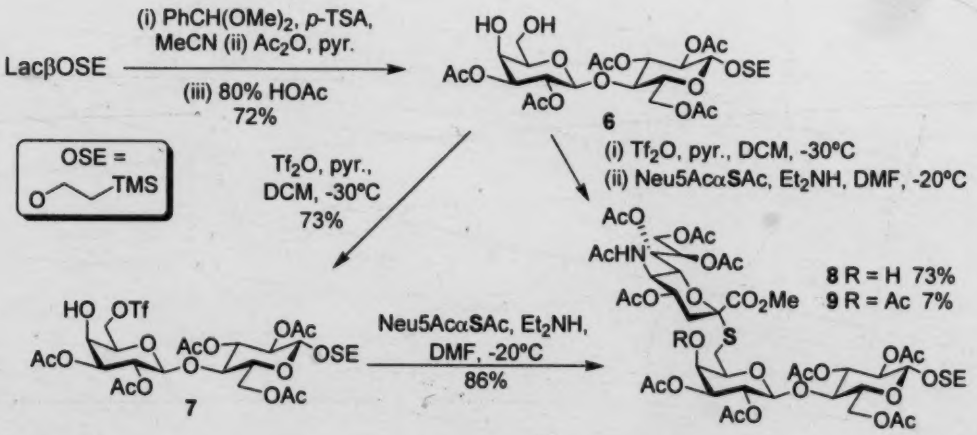
target thioglycoside. Characterization of the reaction products was complicated by the use of an anomeric mixture, and reactions at this anomeric centre appeared to contribute to the low yields. Reaction of the bromide with potassium thioacetate yielded the 6'-thioester **5** in over 90% yield.



Scheme 2.4. *Attempted synthesis of α(2→6') thiosialyl lactose by displacement of the 6'-bromide.*

The 2-(trimethylsilyl)ethyl glycosidic blocking group appeared well suited to thiosialoside synthesis (Scheme 2.3, *route b*), and had been employed previously for this purpose by Hasegawa.[115] The lactoside was obtained by reaction of acetobromolactose with 2-(trimethylsilyl)ethanol under Helferich conditions, followed by deacetylation in methanol containing catalytic sodium methoxide.[122] Reaction of the polyol with benzaldehyde dimethyl acetal in acetonitrile, followed by acetylation of the remaining hydroxyl groups and hydrolysis of the cyclic acetal gave the diol **6** in 72% yield (Scheme 2.5). Attempts to selectively iodinate the primary alcohol under Mitsonobu-type[123,124] conditions afforded a mixture of several products, each of the desired mass. Analysis of the ^{1}H NMR data suggested that migration of the 3'-*O*-acetate to the adjacent axial 4'-

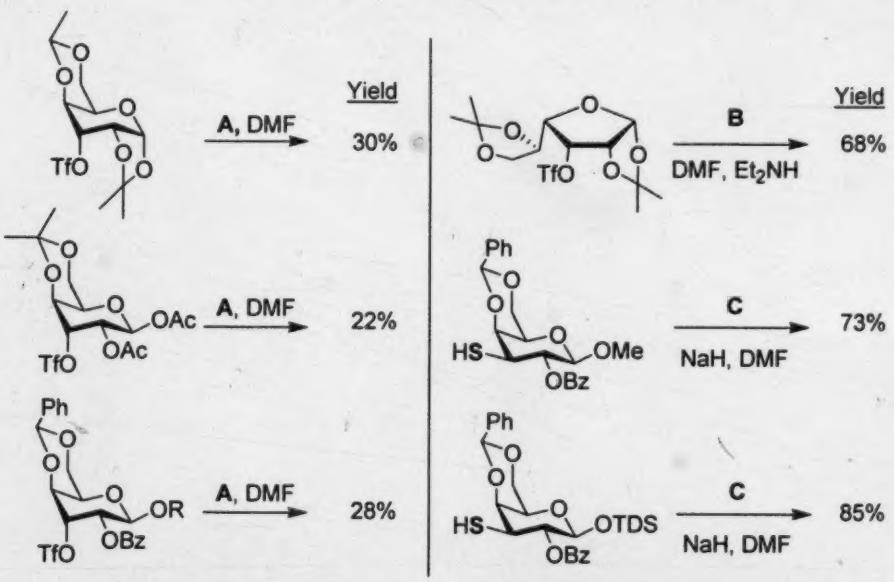
hydroxyl contributed to the poor result. Selective sulfonylation of the primary alcohol **6** was more successful, yielding the triflate **7** and a small amount (<5%) of a product assigned as the ditriflate on the basis of TLC mobility, ^{1}H NMR and mass spectrometry. Reaction of the crude triflate ester under von Itzstein's conditions afforded the desired thiotrisaccharide **8** along with a small amount of the 4′-O-acyl thiotrisaccharide **9**, presumably formed after indirect intermolecular transfer of the acetate from sulfur. When purified on silica gel, the triflate **7** could be characterized and stored at –20°C for several months with minimal decomposition (<10%). Although correct elemental composition values could not be obtained, the more downfield chemical shifts of the H-6a/H-6b resonances relative to the diol and the appearance in the ^{13}C NMR spectrum of a quartet corresponding to the trifluoromethyl carbon provided strong support for this structure. The observation of substantial degradation of the product during chromatography favoured its direct use without purification.



Scheme 2.5. *Synthesis of a protected α(2→6′) thiosialyl lactoside.*

2.2.3 Synthesis of α(2→3) linked thiosialyl galactosides and lactosides

The Neu5Acα(2→3[S])Gal linkage has been synthesized previously from protected gulose derivatives.[125-128] It is clear from literature reports that in contrast to formation of the α(2→6) linked thiosialosyl linkages, anomeric *S*-alkylation does not provide high yields of the thiodisaccharide. Attempts to utilize diethylamine for *in situ*



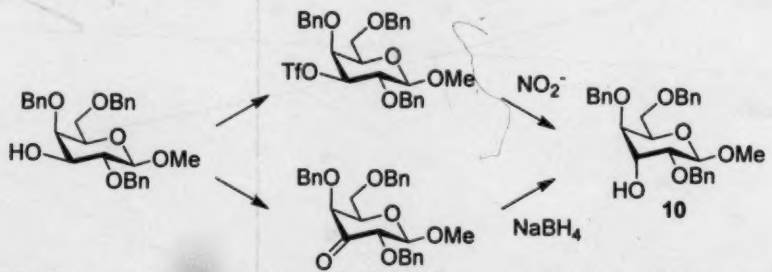
Scheme 2.6. *Reported syntheses of S-linked Neu5Acα(2→3)Gal.*

generation of the thiolate led only to elimination of the leaving group in pyranose systems.[126] Instead, synthesis of the carbohydrate thiol followed by reaction with the halogenose seems preferable. An exception is evident in the reported synthesis of a 3-thiogalactofuranose containing disaccharide, in which diethylamine promoted anomeric

S-alkylation was employed.[128] Among the reported syntheses of this unit only the methods of Field and Schmidt merit serious attention, though the former publication described significant difficulty during deprotection and conversion of the galactosyl residue to the pyranose from the furanose form following establishment of the thioglycosidic linkage.[126,129]. The most efficient route to the target disaccharide, reported by Schmidt, involved synthesis of the D-guloside from D-galactose, inversion of configuration at C-3 of the guloside to give the 3-thiogalactoside, and reaction of the thiol with the sialosyl chloride **C**[130] (Scheme 2.6).

Our intended synthesis also involved epimerization of the 3-OH of a galactoside to the *gulo*-configured alcohol, followed by activation and displacement with a thioacetate nucleophile. Ominously, some analogous though poorly identified reaction schemes were reported to have failed.[129,131] Methyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranose was available from the methyl glycoside in only three steps, and served as a convenient starting point to test a potential synthetic strategy (Scheme 2.7).[132,133] Although we had from the outset sought to avoid benzyl ether protection, there was some question as to which protecting groups were best suited to the double inversion chemistry under investigation. In any case, removal of benzyl groups by dissolving metal reduction was known to be compatible with thioglycosidic linkages in some cases.[134] Treatment of the galactoside with triflic anhydride and pyridine yielded a single product, presumably the triflate, by TLC. Although numerous sulfur nucleophiles are suitable for reaction with carbohydrate sulfonates, fewer of the more basic oxygen nucleophiles are appropriate for this type of reaction. The nitrite anion serves as a hydroxyl surrogate in nucleophilic displacement of sulfonates and has found use in carbohydrate

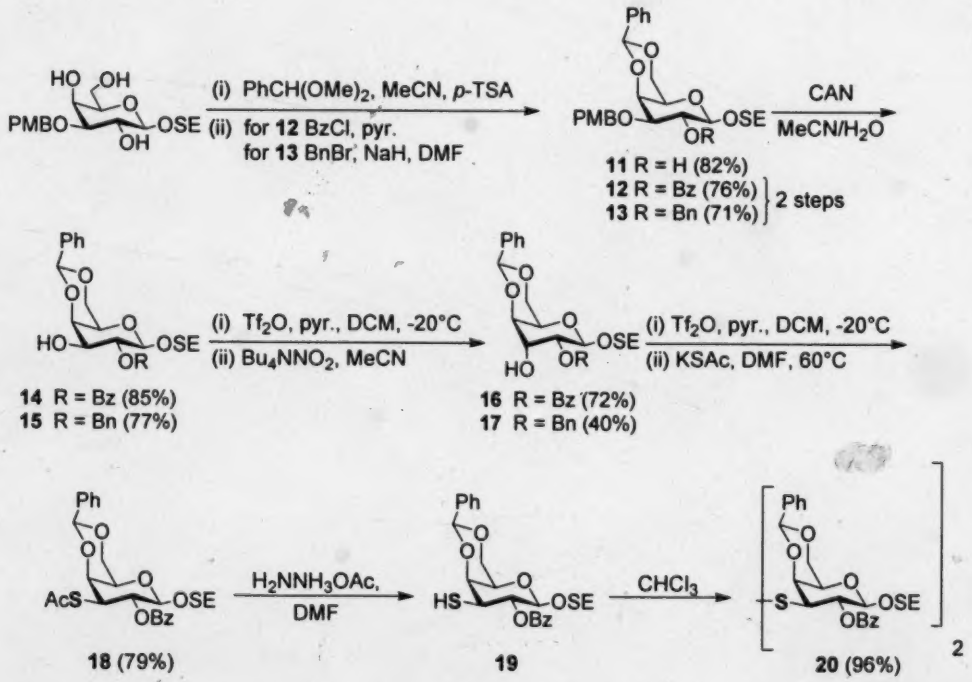
chemistry.[135,136] The nitrite ester initially formed undergoes attack by a second molecule of nitrite, subsequently yielding the alcohol.[137] Treatment of the triflate with tetrabutylammonium nitrite unfortunately gave a complex mixture of products, from which the guloside **10** could be isolated in only (<20%) yield. Attempts to epimerize the alcohol by other methods were also unsuccessful. An oxidation-reduction sequence involving the α-glycoside had previously been reported to afford good yields (70%) of a mixture that heavily favoured the *gulo*- over the *galacto*- epimer (9:1).[131] The facial selectivity in the sodium borohydride reduction was clearly influenced by the anomeric stereochemistry, as in our hands subjection of the β-galactoside to the same conditions afforded a 2:5 mixture of the *gulo*- and *galacto*- configured alcohols.[131] A synthesis that more closely paralleled the earlier report of Schmidt was next explored.



Scheme 2.7. *Attempted epimerization of a benzyl protected galactoside to a guloside.*

The 2-(trimethylsilyl)ethyl protecting group was deemed suitable for protection of the reducing terminus, as judged by its prior utility in the synthesis of α(2→6′) thiosialyl lactose. The use of a transient stannylidene acetal is an established method for regioselective modification of the 3-OH of galactosides.[138] We elected to install a *p*-methoxybenzyl ether at this position according to a literature protocol (Scheme 2.8).[139] Protection of the 4,6-diol as a benzylidene acetal gave the alcohol **11**, which could be isolated in 82% yield. Benzylation or benzoylation of the crude product of this reaction

yielded the fully blocked galactosides **12** and **13**, each in excess of 70% yield. Removal of the substituted benzyl ether with ceric ammonium nitrate yielded substrates **14**[140] and **15**, with which to again test the epimerization strategy.[141,142] Triflation of either product in the usual way at -20°C proceeded with only minor decomposition as judged by TLC. Treatment of the triflates with tetrabutylammonium nitrite yielded the gulosides, although there was a great disparity in yield between the benzoylated (**16**, 72%) and benzylated (**17**, 40%) substrates. The *galacto-* to *gulo-* interconversions were confirmed by a change in the $J_{3,2}$ values in ^{1}H NMR spectra (*galacto-* $J_{3,2}$ ~ 11 Hz; *gulo-* $J_{3,2}$ ~ 3 Hz). As the 40% yield reported here for **17** was by far the best result in a series of test reactions performed on the benzylated alcohol, this galactoside was judged to be a



Scheme 2.8. *Synthesis of a 3-thiogalactoside.*

poor substrate and the synthesis was abandoned at this point. A triflation inversion sequence involving **16** employed potassium thioacetate as a nucleophile and proceeded in reasonable yield to afford the thioester **18**. Treatment with hydrazine acetate effected thioester cleavage to give the thiol **19**, which was slowly converted to the disulfide **20** in a variety of solvents. Thiol and disulfide were differentiated by mass spectrometry, and by slight differences in the chemical shifts of H-3 in the ^{1}H NMR spectrum.

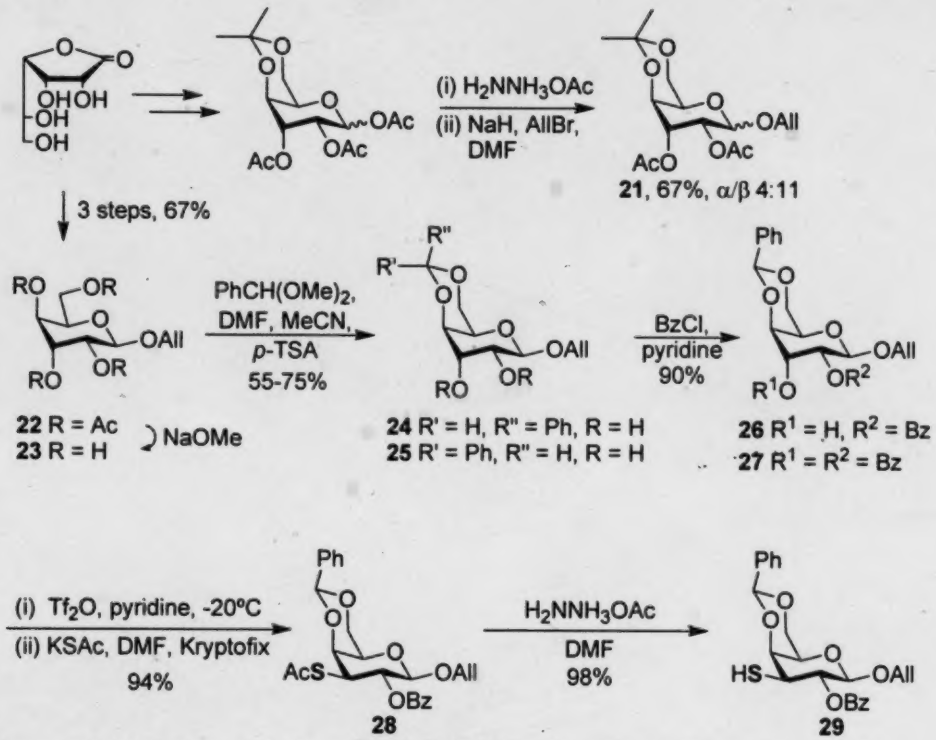
The general inefficiency associated with epimerization strategies as well as some variability in the appearance of side products during the synthesis of guloside **16** from **14** prompted us to examine new strategies for the synthesis of appropriately functionalized D-gulosides. D-Gulose is a relatively expensive monosaccharide,* thus an epimerization strategy appeared warranted. However, D-gulono-γ-lactone is relatively inexpensive,* and upon controlled sodium borohydride reduction, affords D-gulose in high yield.[143] Two potential routes to allyl gulosides, functionalized to allow access to OH-3 were devised based on this initial transformation (Scheme 2.9).

D-gulose is converted to the 4,6-*O*-isopropylidene protected triol or tri-*O*-acetate in high yield.[144] Unfortunately, attempts to achieve anomeric alkylation of the triol or the related 2,3-diacetate by reaction with allyl bromide and base resulted in poor stereoselectivities and moderate yields of **21**.[145,146] Instead, conversion of β-D-gulopyranose pentaacetate to the gulopyranosyl bromide, followed by reaction with allyl alcohol under Helferich conditions and Zemplén transesterification afforded the allyl guloside **23** in 73% yield over 3 steps. The allyl protecting group was selected for its versatility: removal would reveal the hemiacetal central to construction of more complex

* 2005 pricing: D-gulose $500 US/g on 1g scale (Omicron Biochemicals); D-gulono-γ-lactone $6 CDN/g on 5g scale (Aldrich).

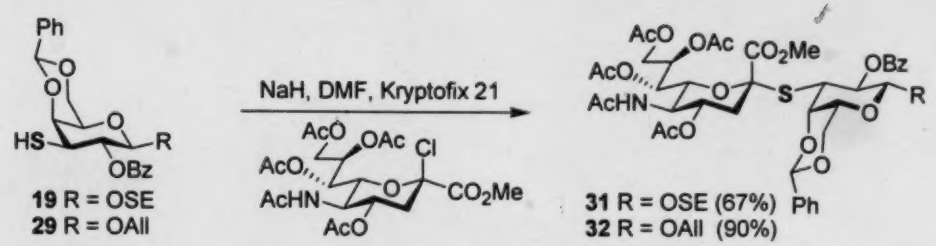
thiooligosaccharides, while elaboration via addition to the terminal olefin would allow preparation of conjugates and clusters. Subsequent installation of a 4,6-benzylidene acetal with benzaldehyde dimethyl acetal in a mixture of DMF and acetonitrile containing catalytic amounts of acid yielded the cis-2,3 diol **24**. When this reaction was conducted using either solvent alone, small quantities of the diastereomeric benzylidene acetal **25** contaminated the reaction mixture. The regiochemistry of acetal formation was established via acetylation of the product diols and analysis of changes in ^{1}H NMR chemical shifts of resonances corresponding to H-2 and H-3, while the configuration of the newly formed acetal was determined by analysis of TROESY spectra. The $^3J_{H1,H2}$ value (4.5 Hz) obtained from the resonance corresponding to H-1 suggested interconversion between $^4C_1$ and $^1C_4$ conformers in the acetylated form of **25**. Not surprisingly, the cis arrangement of the 2,3-diol in gulose resulted in some diacetal formation, and yields for this reaction were somewhat variable. Selective benzoylation of **24** at the more reactive equatorial hydroxyl yielded the axial alcohol **26** in as much as 90% yield, along with a trace of the dibenzoate **27**. The alcohol **26** was converted to the equatorial thioester **28** in high yield by reaction of the corresponding triflate with potassium thioacetate in DMF. De-*S*-acetylation via the action of hydrazine acetate gave the thiol **29** and small amounts of disulfide.[147]

The final step in preparation of the target thiodisaccharide involved coupling of the *galacto*-configured thiolates with the sialosyl chloride (Scheme 2.10). Two difficulties became immediately apparent. Measures were required to minimize disulfide formation during both deprotection of the thioester and the coupling step, and the amount and type of base employed were critical to avoiding elimination of the glycosyl chloride.



Scheme 2.9. *Synthesis of 3-thiogalactoside* ***29*** *from* D*-gulono-γ-lactone.*

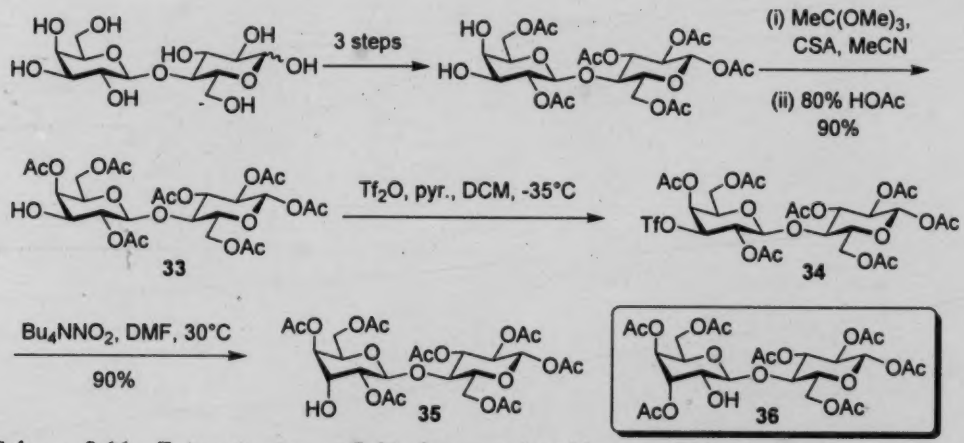
The thioester was cleaved with hydrazine acetate in degassed DMF, normally just prior to use. The thiol (**19** or **29**) was then passed through a short column of silica gel and combined immediately thereafter with sodium hydride and Kryptofix 21 (1,4,10-trioxa-7,13-diazacyclopentadecane) in oxygen free solvent, followed by the glycosyl chloride, to yield the disaccharide (**31** or **32**). The excess of sodium hydride required, and hence the degree to which glycal was formed could be minimized by washing the sialosyl chloride with bicarbonate solution immediately prior to use. The absence of the cryptand was found to decrease yields by approximately 5-10% in two control experiments. The discrepancy between yields realized for disaccharide formation with the allyl and 2-(trimethylsilyl)ethyl glycosides is more likely attributable to experience than reactivity.



Scheme 2.10. *Synthesis of protected α(2→3) thiosialyl galactose.*

No report of the coupling of lactose and sialic acid via a an α(2→3') thioglycosidic linkage had appeared prior to our studies. Two criteria were initially identified for the development of a new synthetic route to α(2→3′) thiosialyl lactose: the trisaccharide should bear a participating group (acetate or benzoate) at *O*-2 of the glucosyl residue to facilitate subsequent glycosylation reactions, and only ester, or ester and acetal protection should be employed after installation of the sulfur atom to simplify deblocking. Access to the 3′-OH of lactose has been achieved by tin-mediated regioselective protection[138] of the 3′-OH of lactosides or indirectly by installation of a 3′,4′-isopropylidene acetal.[148] Baer developed a method to rapidly isolate the 3′,4′-diol by isopropylidenation of the reducing sugar followed by acetylation of the remaining hydroxyl groups and hydrolysis of the cyclic acetal (Scheme 2.7). The major β-isomer could be recrystallized from the mixture following chromatographic isolation of the anomeric mixture, facilitating product identification by ^{1}H NMR in subsequent reactions. Treatment of the diol with trimethylorthoacetate, followed by hydrolysis of the cyclic orthoester intermediate gave the heptaacetate **33**, a crystalline solid. Sulfonylation proceeded smoothly to yield the triflate **34**, which was degraded to some extent by chromatography on silica gel. Displacement of the sulfonate by tetrabutylammonium nitrite proceeded with inversion of configuration to give the axial alcohol **35** in high

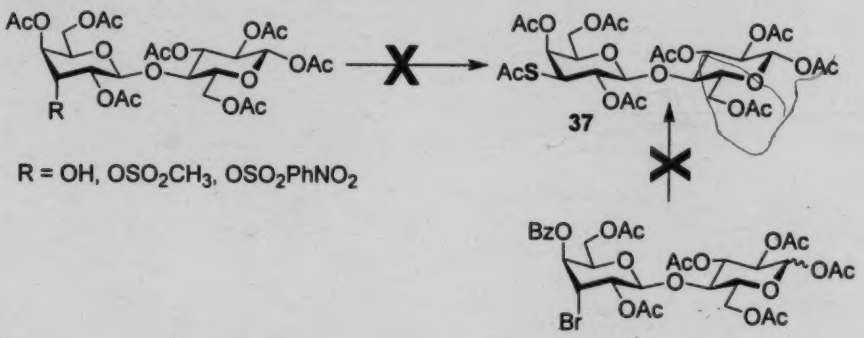
yield. Alternatively, sulfonylation and displacement without an intermediate purification step yielded the same alcohol in 90% yield over two steps. A second product was evident when the displacement reaction was done in either DMF or MeCN at elevated temperatures. Although this product could not be completely separated from **35**, analysis of the ^{1}H NMR spectrum of the mixture suggested the structure to be the regioisomeric equatorial alcohol **36**. Further support for this structure was provided by analysis of the mixture by mass spectrmetry and combustion.



Scheme 2.11. *Epimerization at C-3′ of an acetylated lactose derivative.*

We next sought to install a thiol equivalent in the appropriately configured disaccharide **35** by activation and displacement of the remaining hydroxyl group. The reactivity of the axial alcohol was tempered somewhat relative to its epimer **33**, and higher temperature was required to effect reaction. Unfortunately, at 0°C to RT, the triflate underwent partial decomposition that was intensified by aqueous work up. Our attention turned instead to alternative methods for activation of the hydroxyl and its subsequent conversion to thioacetate (Scheme 2.12). Attempted inversion of the alcohol under Mitsunobu conditions gave only starting material and traces of the elimination

product containing a double bond between C-2′ and C-3′. The mesylate and bromide* were similarly unreactive. Reaction of either with potassium thioacetate in DMF could be monitored by TLC while the temperature was gradually increased. No reaction was evident until the temperature reached 140-150°C, at which time the starting materials decomposed to give a large number of significantly more polar compounds. The *p*-nitrophenyl sulfonate leaving group was expected to exhibit higher reactivity than the mesylate and bromide, but decomposition at high temperatures again occurred.

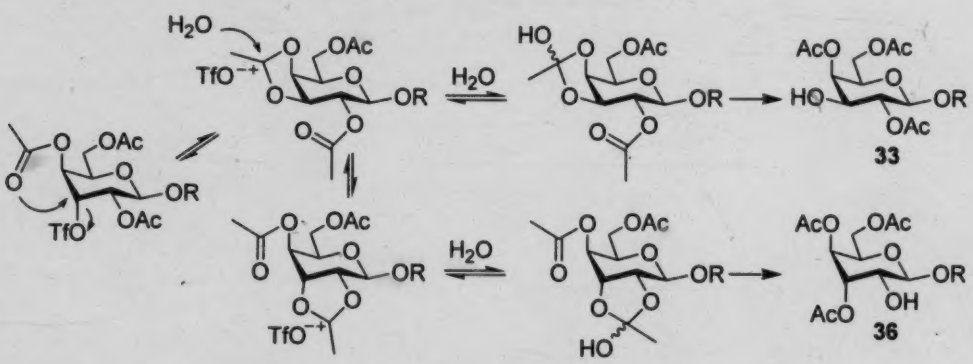


Scheme 2.12. *Potential precursors to protected 3′-thiolactose.*

Given that the axial triflate was at least reactive at lower temperatures, we again turned our attention to its preparation and displacement. By applying a large excess of triflic anhydride (8 eq.) and excess pyridine (12 eq.) at –10°C, the triflate could apparently be generated without significant decomposition as judged by TLC. Exposure to water during aqueous work-up resulted in extensive degradation. Nonetheless, the crude triflate and products of its decomposition were treated with potassium thioacetate in DMF, and a small amount (<15%) of the target material **37** was isolated. The major product of the reaction (~45%) was determined to be its synthetic precursor, the

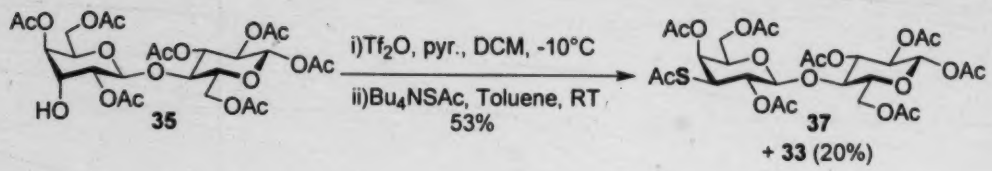
* The bromide was a gift from Dr. Chang-chun Ling.

equatorial alcohol **33**. One can envision its formation via neighbouring group participation followed by hydrolysis of the resulting orthoacid. The simultaneous isolation of a small amount of the 2′-alcohol **36** lends credence to this suggestion. A potential route for the formation of **33** and **36** is suggested (Scheme 2.13). Similar intramolecular displacements of triflate by adjacent acyl groups to give acyloxonium intermediates have been identified and utilized in targeted synthesis.[149] In this case, the acidity of pyridinium triflate must be sufficient to effect rearrangement of the orthoacid intermediate. It is also possible that thioacetate, rather than water, interacts with the acyloxonium intermediate and is expelled on rearrangement.



Scheme 2.13. *Proposed route for formation of **33** and **36** via acyloxonium ion intermediates.*

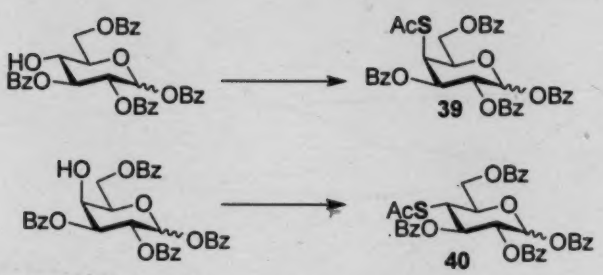
It was subsequently determined that the use of ice water during work up followed by extensive drying of the organic extracts minimized the formation of **33** and **36**. However, yields remained unsatisfactory. Eventually, the combined use of tetrabutylammonium thioacetate as nucleophile,[150] and toluene as solvent increased the yields of the target **37** to over 50% and suppressed (<20%) formation of the equatorial alcohol **33**, provided that the solution of triflate was not concentrated entirely after workup (Scheme 2.14).



Scheme 2.14. *Synthesis of 3'-thiolactose peracetate.*

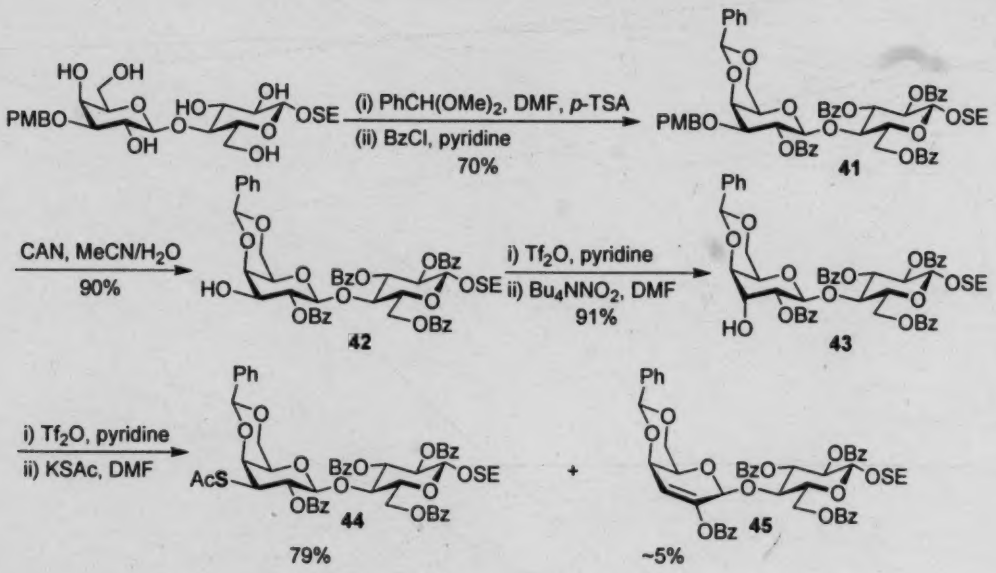
Efforts to deprotect the thioacetate and elaborate the molecule to the thiotrisaccharide were frustrated by the parallel reactivities of the anomeric acetate and thioester. Established methods for the selective deprotection of thioesters in the presence of acetate include the use of hydrazine acetate,[147] cysteamine,[151] sodium methanethiolate,[152] and pyrrolidine.[153] In each case, significant degradation of the anomeric functionality was observed concomitantly. The anomeric acetate could be removed efficiently without affecting the thioester (*vide infra*). However, glycosidation or protecting group exchange prior to coupling with Neu5Ac would drastically diminish the efficiency of this route. Instead, we elected to devise a new synthesis beginning with a lactoside.

To investigate whether acyl migration/participation would be a general feature of displacement reactions on acylated substrates (see Schemes 2.11 and 2.13), two other systems were evaluated (Scheme 2.15). While the 1,2-*cis* arrangement of the triflate and 3-*O*-benzoate in the galactosyl precursor[154] to **40** would likely preclude participation, the 1,2-*trans* diequatorial *gluco*- precursor[155] to **39** is better suited to this type of side reaction upon triflation. The 4-thiogalactose and 4-thioglucose derivatives **39** and **40** were both obtained in high yields without evidence of participation, even at room temperature. It is uncertain whether acetate protecting groups would have fared as well.



Scheme 2.15. *Preparation of protected 4-thio-glucose and 4-thio-galactose.*

The thioganglioside targets **1** and **2** would ideally be derived from the same precursors. That is, *S*-linked GM_2 should be accessible from *S*-linked GM_3. To this end, a further constraint was placed on our previous protecting group strategy: in addition to the installation of a base stable glycosidic protecting group, the 4′-hydroxyl was required to be available without complication following synthesis of the thiotrisaccharide. The 2-(trimethylsilyl)ethyl glycoside was again selected as a starting point owing to its established compatibility with the intended chemistry. Protection of the 3′,4′-diol as an isopropylidene acetal is an effective method for the isolation of these hydroxyl groups. However, a 4′-OH protecting group that would with certainty not interfere with the triflation-inversion chemistry envisioned for the 3′-OH, and that would be compatible with the desired thioglycosidic linkage at the deprotection stage was not immediately apparent. Instead, we chose to employ a 4′,6′-benzylidene acetal in conjunction with tin-mediated regioselective protection of the 3′-position as a *p*-methoxybenzyl ether (Scheme 2.16). Given our experience with the synthesis of thiosialyl galactosides (**31** and **32**), we felt confident of protecting group compatability.

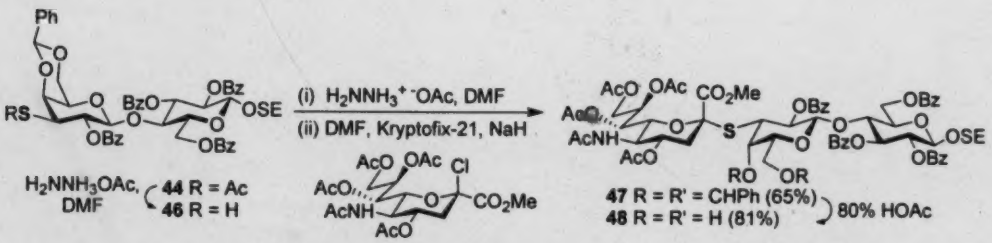


Scheme 2.16. Synthesis of protected 3′-thiolactoside **44**.

2-(Trimethylsilyl)ethyl 3′-*O*-*p*-methoxybenzyl lactoside was available in 6 steps from lactose.[156] Installation of a benzylidene acetal into this substrate proved unexpectedly difficult (Scheme 2.16). Product yields were variable and careful monitoring of the reaction mixture was necessary. Following benzylidenation, benzoylation of the crude product afforded the fully blocked lactoside **41**. Cleavage of the substituted benzyl ether with ceric ammonium nitrate proceeded smoothly to yield the crystalline alcohol **42** in 90% yield.[141,142] At this stage we sought to apply the triflation-double inversion sequence described earlier. Sulfonylation of the alcohol **42** and displacement by nitrite yielded the axial epimer **43**. A second triflation-inversion sequence provided the desired 3′-thiolactoside **44**. Both substitution reactions proceeded in high yields (90%, 79%) after extensive optimization. Despite the enhanced stability of the benzoyl group relative to acetate, migration of the aryl ester was at times observed

during epimerization of the alcohol, as reported for the analogous peracetylated system (**34** → **35** → **36**, Scheme 2.11). Careful temperature control, newly purchased potassium thioacetate (Aldrich), and the use of well dried and degassed solvent helped suppress formation of the 2′,3′-elimination product **45**. It was also found that an isopropylidene acetal could be used in place of the benzylidene protecting group.

Preparation of the target thiotrisaccharide **47** from the 3′-thiolactose derivative **44** (Scheme 2.17) proceeded in a comparable fashion to the synthesis of the thiodisaccharides **31** and **32**. Owing to the acid sensitivity of the (trimethylsilyl)ethyl group there was concern regarding its degradation during removal of the benzylidene acetal to enable chemical synthesis of the thiotetrasaccharide analogue **2** of GM_2 oligosaccharide. Fortunately, the acetal could be hydrolyzed under standard conditions in 81% yield to give the diol **48**.



Scheme 2.17. *Synthesis and partial deprotection of α(2→3′) thiosialyl lactose.*

Near the conclusion of this investigation, von Itzstein's group published work that validated many of our conclusions.[157] In their studies toward the synthesis of 3′-thiolactosides, these workers identified requirements for *galacto-*/*gulo-* epimerization, including problems with 2-*O*-benzyl protection, the potential intermediacy of acyloxonium ions, and ultimately, successful reactions with a 4,6-benzylidene acetal in place.

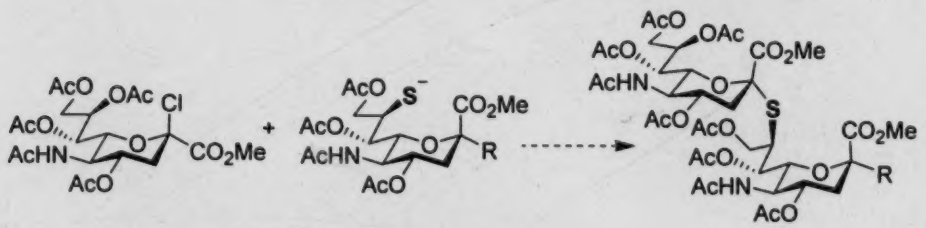
As is evident from the ^{1}H NMR data for 3-*O*-trifluoromethanesulfonyl β-D-gulopyranosides ($J_{1,2} \sim 9$Hz, $J_{2,3} \sim J_{3,4} \sim 3$Hz), these monosaccharides adopt primarily the $^4C_1$ conformation, and as such are predisposed to elimination owing to the 2,3-*trans* diaxial orientation of H-2 and the sulfonate (Figure 2.4). These systems are further destabilized by weakening of the C3-OTf bond due the antiperiplanar arrangement of the C4-O bonding orbital and the C3-OTf antibonding orbital.[128] The significance of this stereoelectronic effect may provide a partial explanation for apparent differences in the stability of the 4-*O*-trifluoromethanesulfonyl galactosyl precursor to **40** and the *gulo*-configured triflate derived from **35**. The galactosyl compound is also predisposed to elimination owing to the 1,2-*trans* arrangement of the triflate and H-5, but this pathway was not observed. The role of 4,6-*O*-benzylidene or isopropylidene cyclic acetals in the success of displacements at *C*-3 of gulosides is unclear. The fused ring system would be expected to hinder flattening of the pyranose ring requisite in S_N2 displacements, but would also preclude neighbouring group participation. Owing to its locked conformation, the cyclic acetal would likely incur less steric interference on the approaching nucleophile than would a flexible acetyl or benzyl group. Ultimately, the success of this strategy may lie primarily in the electronic properties of the acetal, intermediate between electron rich benzyl and electron poor acetyl substituents.

Figure 2.4. *Facile elimination in gulopyranosides.*

2.2.4 Attempted synthesis of 8-thio Neu5Ac

The α(2→8) disialosyl unit is a component of a number of gangliosides. Chemical synthesis of this linkage has proven extremely challenging owing to the unusually low reactivity of the hydroxyl group at C-8. Steric factors, interaction with the acetamide, and hydrogen bonding to the C-1 carbonyl have all been suggested to contribute to this problem.[158] Controlled hydrolysis of colominic acid, an α(2→8) linked Neu5Ac polymer is now often used as a means of obtaining the dimer. Following hydrolysis, the disaccharide can be protected and converted into a glycosyl donor, normally a thioglycoside.

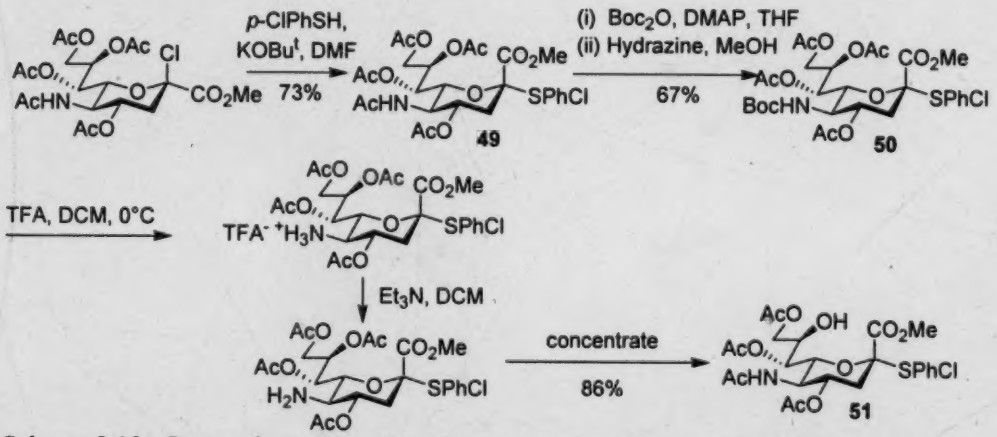
α(2→8) Linked sialosides are found at the non-reducing terminus of oligosaccharide chains and are thus susceptible to degradation by exo-sialidases. We envisioned construction of a hydrolysis resistant thiodisaccharide by means of base promoted *S*-glycosidation, and expect that this coupling would compare favourably to *O*-glycosylation (Scheme 2.18). However, to attempt this reaction we required access to 8-thio Neu5Ac which is likely most readily accessible via a double inversion at C-8.



Scheme 2.18. *Potential route to α(2→8) thiodisialosides.*

A recent report described the regioselective migration of an ester protecting group from O-8 to nitrogen during zinc/acetic acid mediated deprotection of a Neu5Troc derivative.[159] It seems clear that this type of migration, if general, would provide an

easy means of accessing the 8-OH of neuraminic acid. We elected to utilize the *p*-chlorophenyl thioglycoside of Neu5Ac as a starting point (Scheme 2.19). The sialosyl chloride was reacted with the *p*-chlorophenyl thiolate in DMF to afford the α-thioglycoside **49**. Although this created the potential for difficulty in activation of the anomeric centre after installation of a second sulfur atom, there is precedent for this type of reaction.[88,160] Reaction with di-*tert*-butyl dicarbonate and DMAP in refluxing THF yielded the *N*-acetyl-*N*-Boc protected derivative, which was immediately subjected to treatment with hydrazine in methanol to give *N*-Boc protected **50** in 67% yield.[161] Removal of the *tert*-butyl carbamate was achieved by treatment with trifluoroacetic acid. ^{1}H NMR analysis of the crude material indicated the presence of only one product in significant quantity, likely the TFA salt. Addition of triethylamine to a solution of the salt in dichloromethane resulted in its neutralization, as judged by the upfield shift (δ 2.84→ δ 2.51) of the resonance corresponding to H-5 in the ^{1}H NMR spectra. Following



Scheme 2.19. *Regioselective O→N acyl migration from O-8 to N-5 of Neu5Ac.*

concentration, the 8-*O*-acetyl group underwent migration to the amine, yielding the target compound **51** in 86% yield. Efforts to invert the configuration of this centre using

sulfonate activation and displacement have thus far met with failure. The possibility of an oxidation-reduction sequence in place of the initial displacement reaction has not been explored.

2.3 Conclusions

We have developed synthetic routes to protected *S*-linked sialosides. The α(2→3) thiosialyl galactose, α(2→3′) thiosialyl lactose, and α(2→6′) thiosialyl lactose building blocks were designed with elaboration to larger oligosaccharide structures and glycolipids in mind. Once deprotected, these glycosides are potential acceptors for glycosyltransferases and thus may be precursors to analogues of higher gangliosides such as GM_2.

Chapter Three

Chemical and Chemoenzymatic Synthesis of *O*- and *S*-Linked Ganglioside Analogues and Fragments Thereof for the Synthesis of Glycoprotein Conjugates

3.1 Introduction

Determination of the magnitude of an immune response to *S*-linked oligosaccharide conjugate vaccines and measurement of the extent to which *S*-glycoside derived immune sera cross-react with the analogous *O*-glycosides requires both *O*- and *S*- linked GM_3 and GM_2 target ganglioside antigens, in monomeric form and as protein conjugates. Since the Ig binding site is unlikely to embrace the entire oligosaccharide antigen, smaller fragments allow for evaluation of the specificity of sera derived from larger antigens. While soluble carbohydrate inhibitors may offer a more specific definition of antibody binding preferences, these assays consume significant quantities of scarce material. An alternative method to assay binding is to couple the oligosaccharides (screening antigens) to protein, which can be immobilized to evaluate antibody binding. The thiosialoside building blocks described in Chapter 2 were designed to serve as precursors in chemical and potentially chemoenzymatic synthesis of sulfur containing ganglioside analogues suitable for conjugation to protein. A chemoenzymatic approach is favoured for the synthesis of related *O*-glycosides.

A variety of conjugation strategies are employed in the synthesis of glycoconjugate vaccines. Polysaccharides are often isolated from natural sources and thus are not pre-functionalized for incorporation into conjugates. Frequently the aldehyde group on the reducing terminal residue is employed for coupling to amino groups on protein via reductive amination. Although less significant for large polysaccharides containing

multiple repeating units, this methodology results in presentation of the reducing terminal monosaccharide residue in its modified acyclic form, and is thus unsuitable for smaller oligosaccharide immunogens of which this single residue may comprise a substantial portion. Synthetic oligosaccharides may be conjugated to protein via reductive amination strategies. Typically, this is achieved by ozonolysis of an olefin containing anomeric protecting group, such as an allyl or pentenyl protecting group, followed by reaction with lysine residues on the protein, and sodium cyanoborohydride reduction of the resulting imine.[162] Ozonolysis has also frequently been applied to gangliosides of natural and synthetic origin.[35] In recent years a variety of homo- and hetero-bifunctional cross linking agents have gained prominence as linkers for preparation of conjugate vaccines. These reagents, many of which are now commercially available, are most commonly reactive with amines and thiols.[163]

Gangliosides are amphipathic molecules. The hydrophilic portion is exposed at the cell surface, while the hydrophobic alkyl substituents are buried within a lipid membrane. Although the exact nature and length of the alkyl chains have been demonstrated to play a crucial role in the activity of various ceramides and glycolipids, recognition in the context of the immune system is dependent on the exposed polar substituents, primarily the carbohydrate.[164] However, the stereochemical features of the lipid portion of the gangliosides, namely the trans-amido alcohol and double bond, may impact the overall conformational features of the oligosaccharide.[165]

The synthesis of gangliosides with full-length ceramide substituents seems unnecessarily laborious in the context of conjugate vaccines. Instead, we have chosen to employ a truncated ceramide aglycon that incorporates the essential stereochemical

features of naturally occurring ceramide, but which is designed to facilitate coupling of the oligosaccharide to protein carriers. This strategy is based on a five carbon azidosphingosine homologue that has previously found use in the Bundle group (Figure 3.1).[166,167] Several recent literature reports describe similar sphingosine and ceramide analogues.[168-170] In addition to the flexibility afforded to the protein coupling strategy by the presence of the terminal alkene and latent amino group, an additional advantage is conferred by the well established superiority of azidosphingosines to ceramides as glycosyl acceptors.[171] One can envision elaboration of the azidosphingosine following glycosylation by either addition of a thiol such as cysteamine to the double bond, or by reduction of the azide followed by *N*-acylation. The potential of these homologues for extension to naturally occurring sphingosine or ceramides by olefin metathesis has also been demonstrated.[169,170]

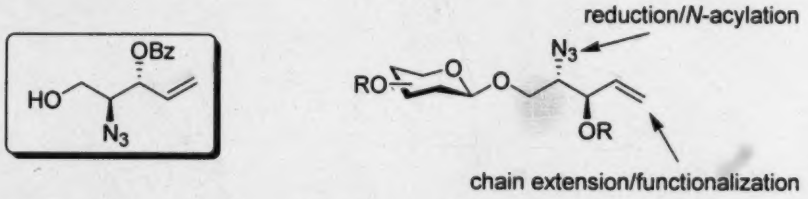


Figure 3.1. *Truncated azidosphingosine (2S,3R) 2-azido-3-O-benzoyl-pent-4-ene-1,3-diol provides flexibility in coupling to proteins.*

3.2 Chemical and enzymatic strategies for the synthesis of gangliosides.

The chemical synthesis of some complex gangliosides has been attainable for nearly two decades, although this work has been primarily the domain of specialist research groups.[172-175] The chemical synthesis of a generic ganglioside can be subdivided into three steps: the synthesis of the oligosaccharide backbone, the introduction of sialic acid(s), and coupling of the oligosaccharide and lipid

aglycon.[172,174] Several important synthetic difficulties have been identified. The introduction of sialic acid(s) into the oligosaccharide backbone remains problematic in terms of both yield and stereoselectivity.[158,176] Owing to the low reactivity and susceptibility to elimination of sialosyl donors, particularly at sites of vicinal branching, the sialosyl residue must be installed at an early stage. Pairing of the carbohydrate and lipid aglycon is generally achieved by coupling of the intact ganglioside oligosaccharide with an azidosphingosine acceptor. Introduction of sphingosine or ceramide at an earlier stage may result in decreased reactivity of the oligosaccharide during subsequent glycosylation reactions. Azidosphingosine has proven to be a superior acceptor as the reactivity of the primary alcohol in sphingosine or ceramide is diminished by the formation of a hydrogen bond with the amide proton (Figure 3.2).[177]

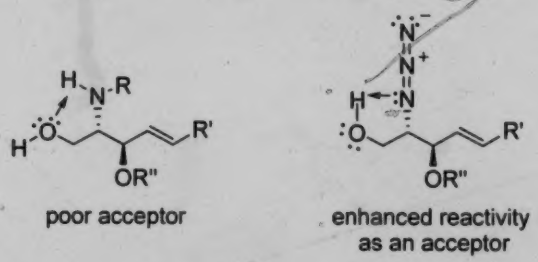


Figure 3.2. *Interactions (dis)favouring glycosidation of ceramide, sphingosine or azidosphingosine.*

Several other features of a general ganglioside synthesis are noteworthy. It has been shown that the 2′,3′,4′-*lacto*-triol is in most cases the best acceptor for chemical synthesis of the Neu5Acα(2→3)Galβ(1→4)Glc trisaccharide, and preparation of this appropriately protected lactoside requires multistep construction from the constituent galactosyl and glucosyl residues rather than the readily available disaccharide.[178] Furthermore, benzyl ethers or other electron rich protecting groups on the acceptor are

required to achieve even moderate yields in glycosylation reactions. Yields for both the sialylation and subsequent glycosylation reaction are low, rarely exceeding 50%.

Chemoenzymatic synthesis of oligosaccharides has proven an attractive and often more efficient alternative to chemical routes.[129,179,180] In particular, glycosyltransferases catalyze the shift of a monosaccharide from a glycosyl donor (usually a sugar nucleotide) to a glycosyl acceptor with a high degree of regioselectivity, stereoselectivity and catalytic efficiency. These enzymes are often obtained by isolation or cloning from mammalian sources and may be expressed in systems including insect cell lines, yeast, and bacteria. More recently, enzymes from non-mammalian sources have enjoyed growing prominence.[181-184] Mammalian glycosyltransferases are frequently type II transmembrane proteins, localized to the Golgi apparatus. These proteins are difficult to purify and require detergents to retain activity and solubility. Most bacterial systems lack the enzymes required for normal glycosylation of mammalian proteins, a feature that is essential to their correct folding and stability. In contrast, many recombinant transferases of bacterial origin have proven to be soluble and stable after expression in *E. coli*, making their use amenable to scale up. The generally relaxed substrate specificity of microbial enzymes is of crucial importance in the synthesis of oligosaccharide analogues.[184] This is true not only of the glycosyltransferases, but also the enzymes involved in generation of sugar nucleotide donors and their sugar 1-phosphate precursors.[185-188]

The core lipooligosaccharides (LOS) of the mucosal pathogens *Campylobacter jejeuni* and *Neisseria meningitidis* contain oligosaccharides that mimic ganglioside structures. Identification and cloning of the glycosyltransferases and associated enzymes

responsible for LOS biosynthesis has provided the synthetic chemist with a suite of relatively stable catalysts analogous to mammalian ganglioside glycosyltransferases.[183,189] Over expression of these enzymes along with glycosyl kinases and nucleotidyltransferases in *E.coli* with simultaneous suppression of degradative enzyme production has proven to be a popular method for the synthesis of gangliosides on a large scale.[190-192] While highly efficient, the use of cellular systems is limited by the restricted uptake of saccharide acceptors by the bacteria.[193] To circumvent these difficulties, the crude or purified enzyme preparations can be used individually or in concert in cell free systems.

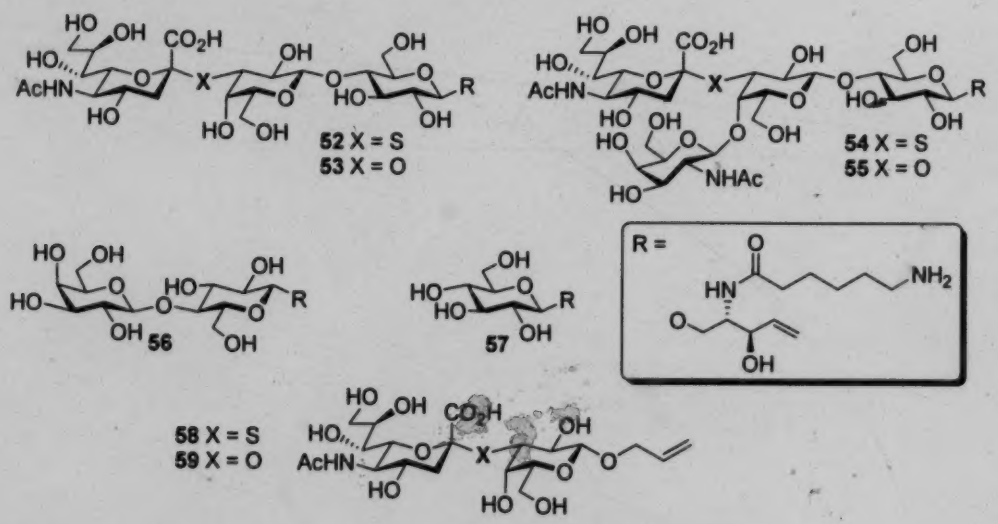


Figure 3.3. *Target oligosaccharides for use in glycoprotein conjugates.*

3.3 Target molecules.

The (thio) oligosaccharides designed to couple to protein for use as immunogens and screening antigens are depicted in Figure 3.3 (**52-59**). Molecules bearing a primary amine were designed to facilitate coupling via widely available homobifunctional linkers to the ε-amino functions of lysine residues exposed at the surface of carrier proteins. A

functionalized *N*-acyl chain was selected as an attachment point on the glycosyl pseudo-ceramides **52-57**. Although conjugation could also be brought about via elaboration of the sphingosine double bond,[167] the depicted strategy offered the benefit of a minimal number of late stage transformations and, although of unknown significance, left the olefin intact.[164] The allyl terminated Neu5Acα(2→3)Gal disaccharides **58** and **59** were identified as molecules that might serve dual purposes. After elaboration of the olefin moiety, the disaccharides would be suitable for conjugation, while without modification they could serve as soluble inhibitors.[194,195]

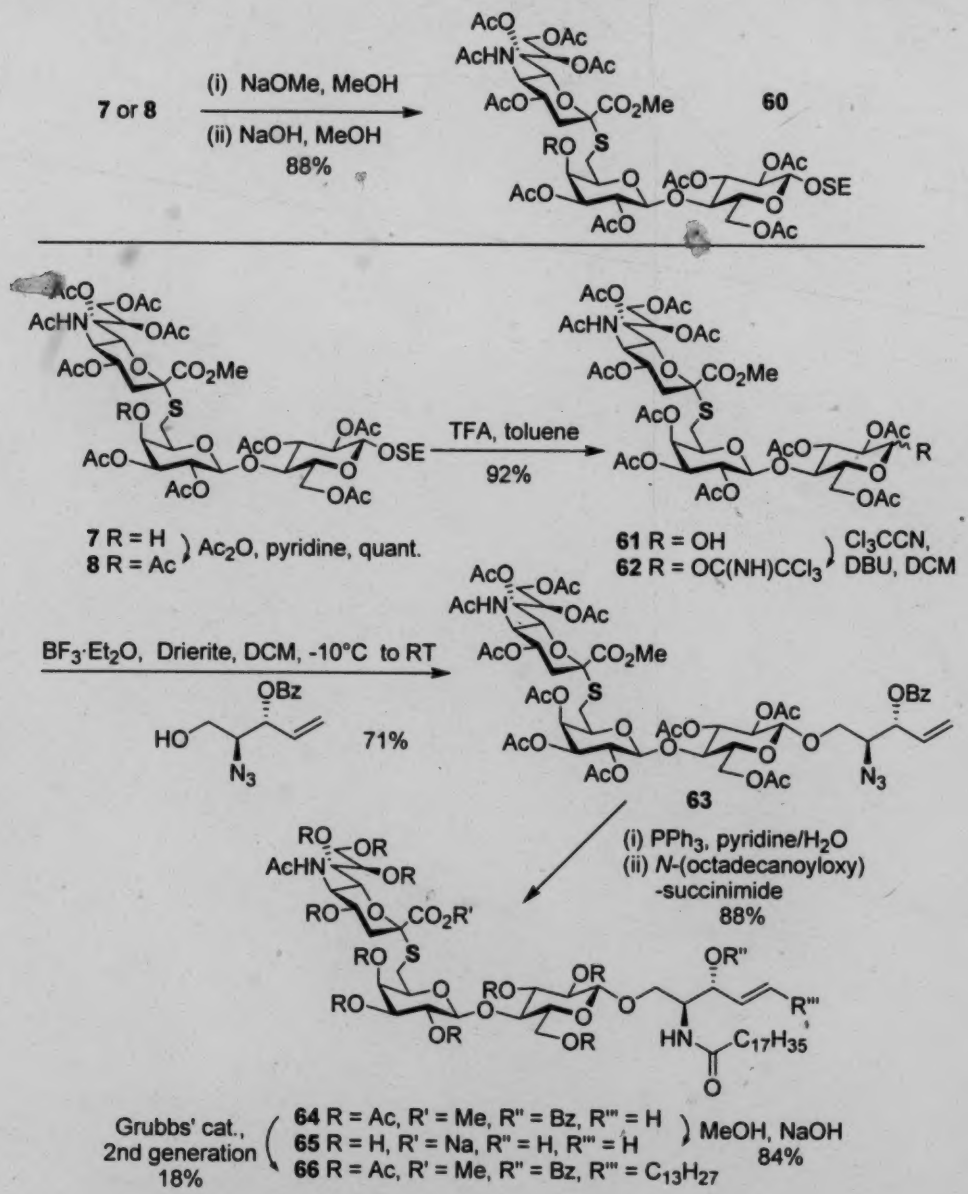
3.4 Synthesis of *S*-linked glycosphingolipid analogues from protected thiosialosides.

Several routes to protected thiooligosaccharides comprising α(2→3) thiosialyl galactose and α(2→3′) thiosialyl lactose were described previously. These compounds were designed for elaboration to the glycosyl ceramides and coupling to protein following deprotection.

3.4.1 Elaboration of α(2→6′) thiosialyl lactose.

Our initial efforts again focussed on elaboration of the more readily available α(2→6′) linked thiotrisaccharide prior to investigation of the primary target structures (Scheme 3.1). Before coupling the trisaccharide to the truncated azidosphingosine analogue, we examined its tolerance toward the intended deprotection conditions. Treatment of trisaccharides **7** or **8** with sodium methoxide, followed by addition of a small quantity of water to effect saponification of the methyl ester afforded the deprotected α(2→6′) thiosialyl lactoside **60** in good yield. Encouraged, we proceeded with efforts to synthesize the glycosylceramide analogue. Acetylation of the lone hydroxyl group of **7**, followed by removal of the anomeric protecting group with trifluoroacetic acid provided

the thiotrisaccharide hemiacetal **61**[115] (Scheme 3.2). Conversion of the reducing sugar to the trichloroacetimidate **62**[115] and glycosidation of (2*S*,3*R*)-2-azido-3-*O*-benzoyl-



Scheme 3.1. *Synthesis and deprotection of S-linked ganglioside analogues containing an α(2→6') thiosialosyl linkage.*

pent-4-ene -1,3-diol* proceeded in good yield to afford the truncated glycosyl azidosphingosine analogue **63**. Subsequent reduction of the azido group under Staudinger conditions and acylation of the crude product with *N*-(octadecanoyloxy) succinimide[196] gave the protected ganglioside analogue **64** which, following Zemplén transesterification and saponification of the methyl ester, yielded the thioglycolipid **65**. This brief sequence helped to establish the viability of the 2-(trimethylsilyl)ethyl anomeric protecting group, the use of trichloroacetimidate coupling methods with the abbreviated azidosphingosine acceptor, and the use of a one pot reduction-*N*-acylation sequence for conversion of the glycosyl sphingosine to the glycosyl ceramide.

The protected ceramide homologue was a potential substrate for elaboration to the long chain structure by olefin cross metathesis. Treatment of a small amount of **63** with Grubbs' second generation ruthenium olefin metathesis catalyst and pentadecene yielded only small quantities of the desired product **66** after extended reaction times and addition of a near stoichiometric quantity of "catalyst".[197] A single attempt to employ the first generation Grubbs' catalyst or the Hoveyda-Grubbs' catalyst failed to improve upon these results.[198]

3.4.2 Synthesis of *S*-linked GM_3 from α(2→3) thiosialosyl galactose and α(2→3') thiosialosyl lactose.

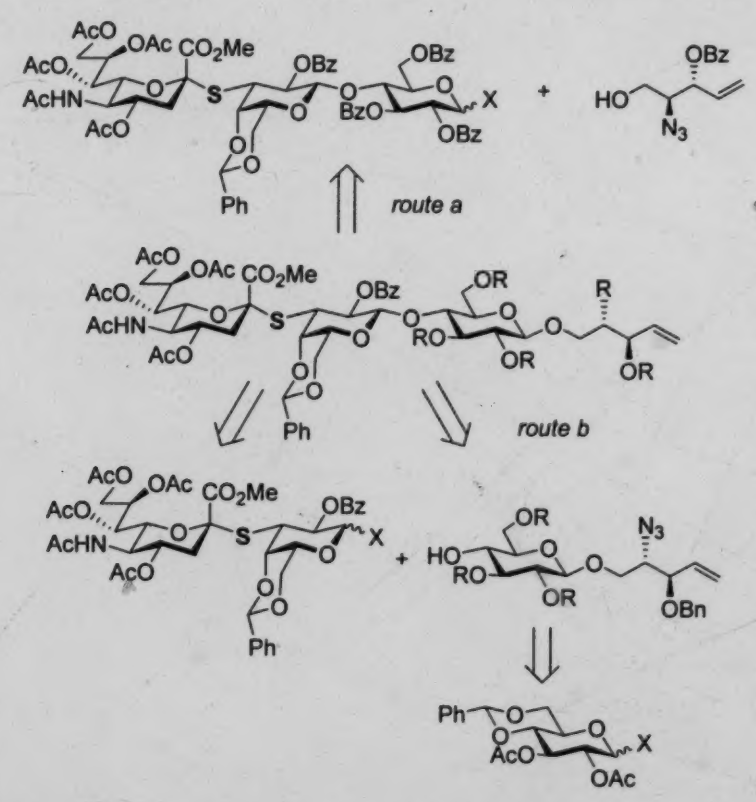
Synthesis of a sulfur containing analogue of GM_3 can be envisaged from either the protected *S*-linked trisaccharide (*route a*) or disaccharide (*route b*), as outlined retrosynthetically in Scheme 3.2. The former intermediate provides a considerably more direct route to the primary target, but this course lacks the flexibility required to obtain

---

* The preparation of this material by Sandra Jacques and Corwin Nycholat is gratefully acknowledged.

oligosaccharide fragments for immunochemical studies. Instead, we favoured coupling of Neu5Acα(2→3[*S*])Gal with a glucosyl ceramide analogue as depicted in *route b*.

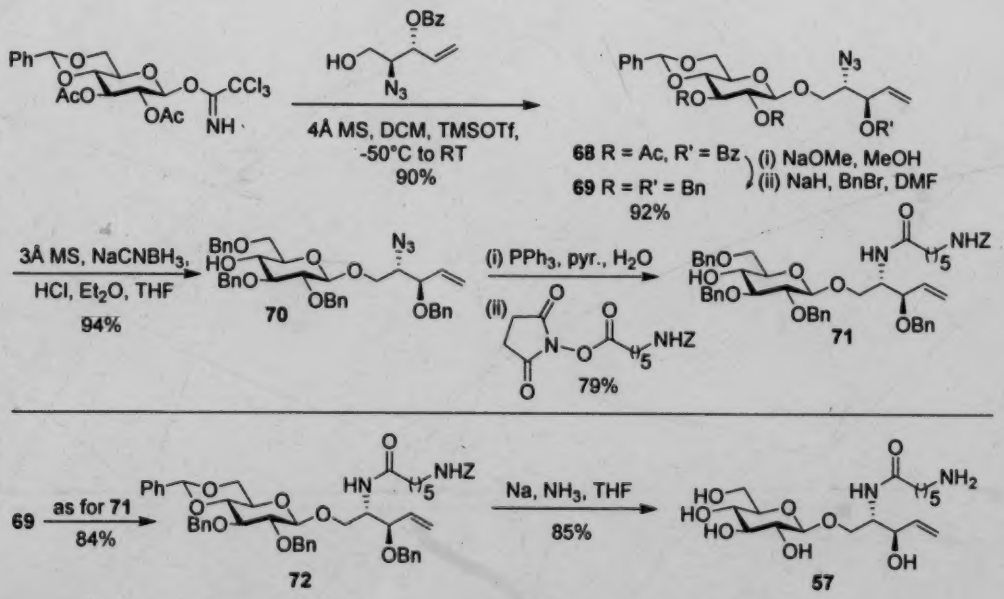
The synthesis of glucosyl azidosphingosine and glucosyl ceramide analogues, protected for use as glycosyl acceptors, is shown in Scheme 3.3. The use of benzyl or other ether protecting groups on glycosyl acceptors serves to activate the hydroxyl group for subsequent glycosylation, in contrast to electron withdrawing protecting groups such as esters. The 4-OH of glucose is known to be relatively unreactive and consequently the



Scheme 3.2. *Retrosynthesis of a protected thio-GM$_3$ intermediate.*

construction of the Galβ(1→4)Glcβ linkage normally requires the use of such a strategy to achieve good product yields. However, the use of a participating group, normally an

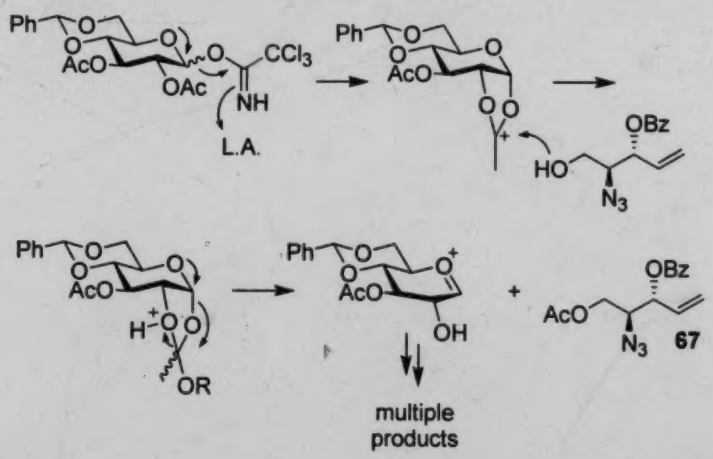
ester, at *O*-2 of the glucosyl residue is also important in stereoselective formation of the 1,2-trans-β-glycosyl linkage. The glucosyl trichloroacetimidate (Scheme 3.3) could be prepared in 4 steps from D-glucose,[199] and was selected with these requirements in mind. Glycosidation of (2*S*,3*R*) 2-azido-3-O-benzoyl-pent-4-ene-1,3-diol proceeded in high yield and exchange of acetate for benzyl protecting groups followed. Acyl transfer was observed in the glycosylation reaction yielding minor amounts (<5%) of the acetylated acceptor **67**, presumably via an intermediate orthoester (Scheme 3.4). Following protecting group exchange, sodium cyanoborohydride mediated regioselective cleavage of the benzylidene acetal **69** furnished the acceptor **70** and a small amount of a side product, likely benzyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (<2%).[200] Reduction of the azide and *N*-acylation of the resultant amine with an *N*-hydroxy succinimide activated ester derived from 6-amino-*N*-carboxybenzyl caproic acid yielded



Scheme 3.3. *Synthesis of protected glucosyl ceramide homologues.*

the alternative acceptor **71**.[201] Protection of the terminal amine as a benzyl carbamate was identified as a means to allow eventual removal of all protecting groups on this fragment in a single step. The fully protected glucoside **72** was obtained by the same chemistry and was subsequently deprotected under dissolving metal conditions to give the glucosylceramide-like amine **57** for use in immunochemical studies.

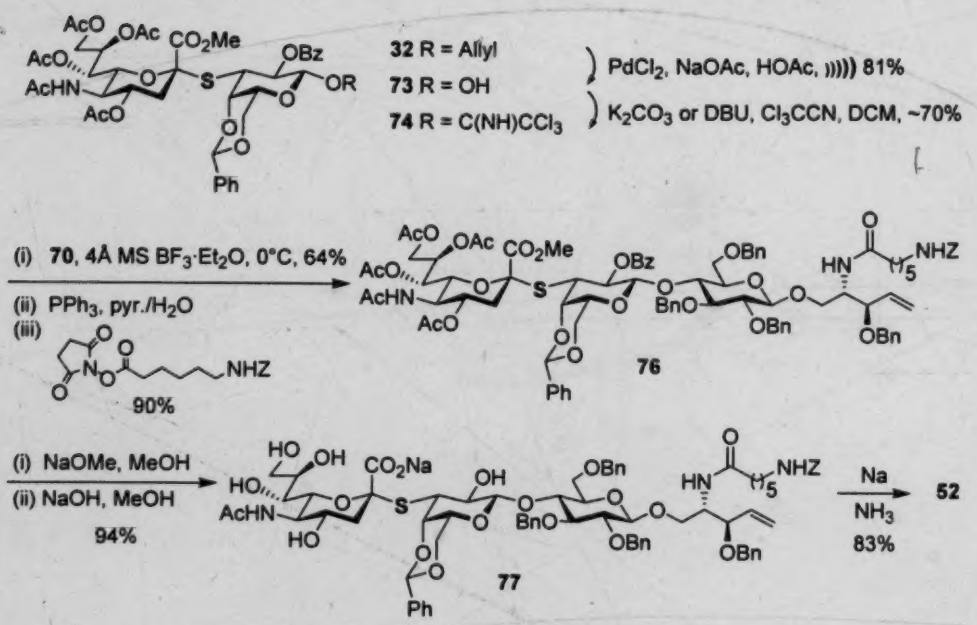
Removal of the allyl protecting group to convert thiodisaccharide **32** into a glycosyl donor for reaction with **70** or **71** proved somewhat problematic (Scheme 3.5). Reaction of **32** with tris(triphenylphosphine) rhodium chloride resulted in minimal consumption of product (<10%).[202] A one-pot deprotection was attempted using



Scheme 3.4. *Proposed mechanism for formation of* ***67*** *by intermolecular acyl transfer.*

palladium chloride in methanol. Under these conditions product decomposition occurred before significant quantities of starting material were consumed. Exchange of methanol for buffered acetic acid[203] gave slightly better yields (~ 40%), but decomposition predominated. Fortunately, a significant rate enhancement was realized upon sonication of the mixture in an ultrasonic cleaning bath (<30 °C), and the disaccharide hemiacetal **73** could be isolated as a mixture of isomers in reasonable yield (81%) after only five

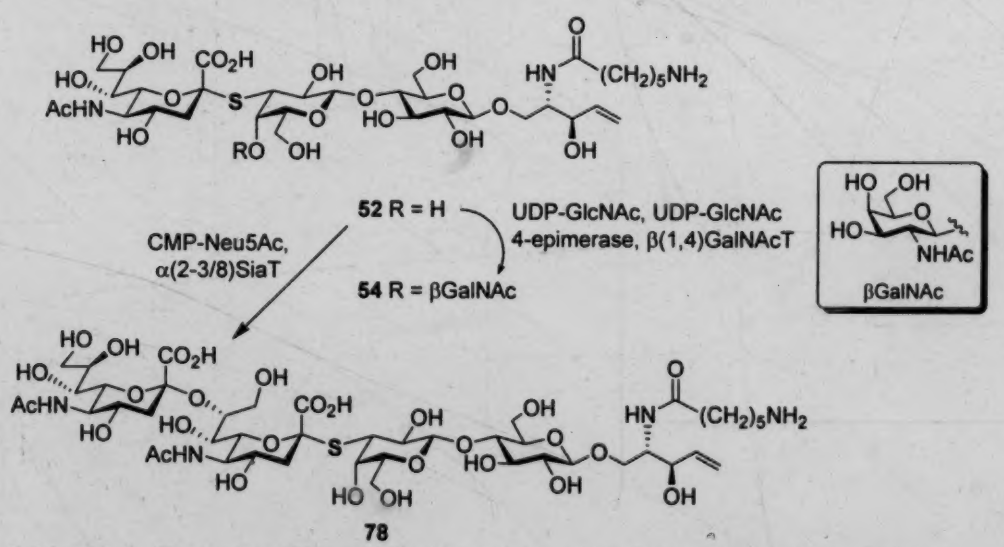
hours. The same product was potentially available from the 2-(trimethylsilyl)ethyl protected disaccharide **19**. However, in either case all attempts to remove the anomeric protecting group prior to removal of the benzylidene acetal were unsuccessful. Conversion of the hemiacetal **73** to the trichloroacetimidate **74** was effected using either potassium carbonate or DBU as base. However, some impurity (<10%) could not be separated from the imidate. Glycosylation of acceptor **70** with the imidate using boron trifluoride diethyletherate or trimethylsilyl trifluoromethanesulfonate as promoter in dichloromethane gave moderate yields of the trisaccharide **75**. Despite all efforts to exclude water from the reaction mixture, a substantial amount of hemiacetal **73** was recovered after workup. One pot reduction of the azide and acylation of the resulting amine with the activated ester[201] yielded the protected GM_3 analogue **76**. Similarly, glycosylation of the *N*-acylated acceptor **71** gave **76** directly albeit in slightly reduced overall yield. Treatment under Zemplén conditions at room temperature removed acetate protecting groups and the benzoate ester was subsequently cleaved upon heating the solution at 55 °C. Addition of water and continued heating gave the salt of the carboxylic acid **77** in 94% yield. The terminal alkene precluded removal of benzyl ether and benzyl carbamate protecting groups by hydrogenolysis over a palladium catalyst. Dissolving metal reduction proved to be a suitable alternative in this case, and the GM_3 analogue **52**, bearing a 1° amine at the *N*-acyl chain terminus was isolated in 83% yield following HPLC. It is noteworthy that substantial losses were incurred when conventional SepPak or C18 silica gel HPLC columns were employed. This problem was overcome by use of a polymeric reverse phase column from Hamilton, or by inclusion of ammonium hydroxide in the mobile phase.



Scheme 3.5. *Synthesis and deprotection of S-linked GM_3 analogue* **52**.

Although the action of glycosyltransferases on modified glycosyl acceptors has precedent, the frequent intolerance of these enzymes towards even minor substrate modifications left the prospect of elaboration of thiosialyl lactosides to GM_2-like tetrasaccharide structures uncertain.[204,205] Despite its thioglycosidic linkage we found that **52** served as an acceptor for glycosyltransferase catalyzed elaboration to the *S*-linked GM_2 analogue **54** (Scheme 3.6). This is a rare example of a Leloir glycosyltransferase mediated glycosylation of a thiooligosaccharide acceptor.[206,207] Bacterial transglucosylases,[208-210] as well as sulfotransferases and acyltransferases from *Sinorhizobium meliloti*,[211] have also been shown to utilize thiooligosaccharides as substrates in some cases. This outcome is noteworthy given that the site of atomic substitution is directly adjacent to the site of glycosylation. The reaction was conducted in the presence of two previously cloned enzymes, a bacterial β(1,4) *N*-

acetylgalactosaminyl transferase (β(1,4)GalNAcT) from *Campylobacter jejuni* serotype O:36,[183] and a UDP-GlcNAc 4-epimerase[212] from the *C.jejuni* NCTC 11168 that catalyzes the conversion of UDP-GlcNAc to UDP-GalNAc.* Treatment of **52** with a cloned bifunctional α(2→3/8) sialyltransferase (α(2,3/8)SiaT) from *C.jejuni* OH4384 and the appropriate nucleotide donor resulted in conversion to the sulfur containing GD_3 analogue **78**.[213] Successful glycosylation of the thiooligosaccharide by two enzymes encourages the conclusion that **52** adopts conformations similar to that of *O*-sialyllactose.

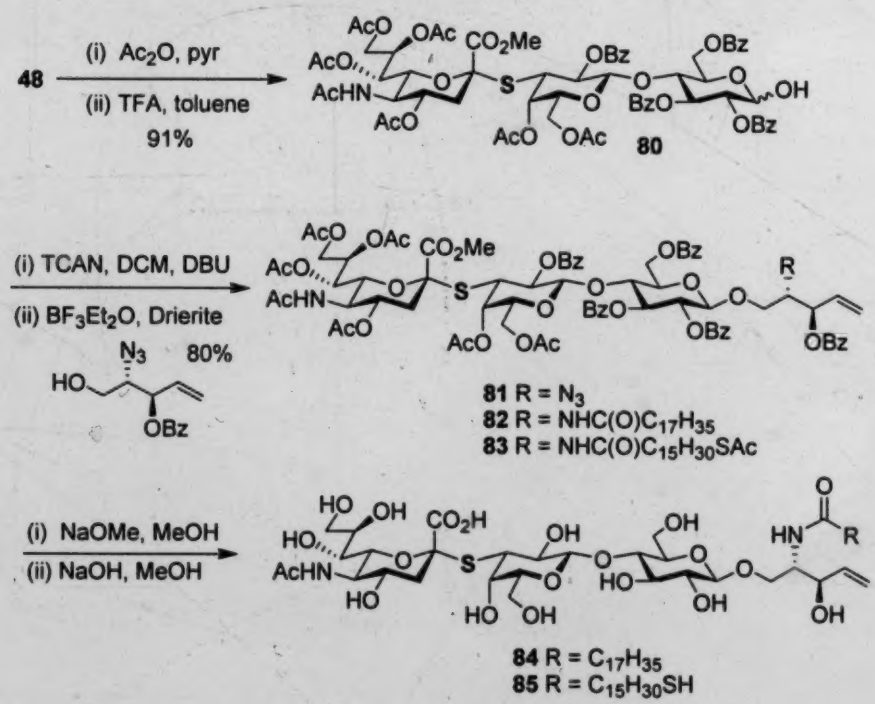


Scheme 3.6. *Enzymatic elaboration of S-GM_3 to sulfur containing analogues of GM_2 (**54**) and GD_3 (**78**).*

The protected *S*-GM_3 trisaccharide **48** could also be elaborated to the glycosyl pseudo-ceramide as depicted in Scheme 3.7. Prior removal of the benzylidene acetal was required to allow for smooth deprotection of the anomeric blocking group with TFA. The 4′,6′-diol formed by acetal hydrolysis of **48** is a potential synthetic precursor to *S*-GM_2 or higher gangliosides following selective protection of the remaining primary

* UDP-GlcNAc $111/g, UDP-GalNAc $1459/0.1g (Sigma, 2005).

alcohol. Efforts to effect the regioselective reductive cleavage of the benzylidene acetal failed, possibly owing to incompatibility of the reducing environment with multiple acyl groups. Protection of the diol in a mixture of acetic anhydride and pyridine gave the diacetate **79**, which was converted to the hemiacetal **80** with trifluoroacetic acid. Glycosidic bond formation was achieved using trichloroacetimidate coupling in a manner analogous to that reported for construction of the α(2→6') thiosialyl regioisomer (Scheme 3.1), with a slight improvement in yield. The azide in **81** could be manipulated using Staudinger chemistry followed directly by *N*-acylation with *N*-(octadecanoyloxy)-



Scheme 3.7. *Synthesis and deprotection of S-linked glycolipids.*

succinimide[196] or *N*-(*S*-acetyl-16-mercaptohexadecanoyloxy)-succinimide[214] to yield either of the long chain ceramide analogues **82** or **83**. Alkane thiols are versatile

functions suitable for coupling of glycolipid to protein or for its immobilization on a gold surface. Deprotection of these compounds proceeded using a sequence of Zemplén transesterification and saponification to yield the GM_3 analogues **84** and **85**.

3.4.3 Synthesis of *O*-linked ganglioside analogues

Mammalian ganglioside biosynthesis is characterized by sequential glycosyltransferase catalyzed addition of activated monosaccharides to a ceramide aglycon. Clones of a number of the analogous enzymes from *C. jejuni* have been made available by the National Research Council's Institute for Biological Sciences, greatly simplifying the synthesis of *O*-ganglioside analogues.* Enzymes could be used without substantial purification, simply by isolation of either the pellet or supernatant following centrifugation of *E. coli* cell culture lysates. A CMP-sialic acid synthetase from *N. meningitidis*, which catalyzes the formation of CMP sialic acid from sialic acid and CTP was also available.[215] Since the activated sugar nucleotide donor is relatively expensive and is unstable, this enzyme was a valuable source of donor for preparative scale glycosylations. Syntheses were conducted on up to one gram of material, which following sedimentation of protein and lyophilization could be used in crude form with an estimated conversion of 90-95%.

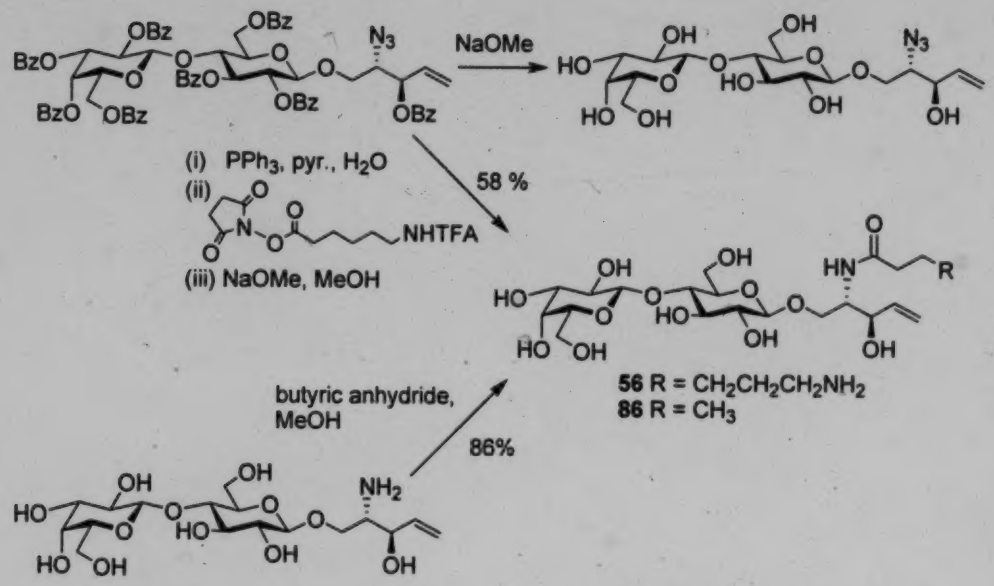
In addition to the amine terminated *O*-ganglioside analogues **53** and **55**, simple alkane functionalized saccharides were required for use in inhibition assays. Our initial efforts focused on the enzymatic elaboration of the known benzoylated and unprotected lactosyl (azido)sphingosine homologues (Scheme 3.8).[166] Although enzymatic reactions with the deprotected lactoside appeared to proceed cleanly by TLC, the absence

---

* The preparation of enzymes by Sandra Jacques, Blake Zheng, and Lisa Pritchard is gratefully acknowledged.

of significant hydrophobic character rendered standard reverse phase purification strategies impractical. To increase the carbon content of the molecules, the azide was reduced and the amine acylated prior to glycosylation. The preparation of lactosyl ceramide analogues **56** and **86** is described in Scheme 3.8. The azide was reduced with triphenylphosphine, and the crude amine reacted with *N*-(*N*-trifluoroacetamido-6-amino-hexanoyloxy)-succinimide.[216] Removal of the trifluoroacetamide and benzoate esters in a single step afforded the amine **56** in 58% yield over three steps. Reaction of the unprotected lactosyl amine[166] with butyric anhydride in methanol provided the analogous *N*-butyl amide **86** in 86% yield.

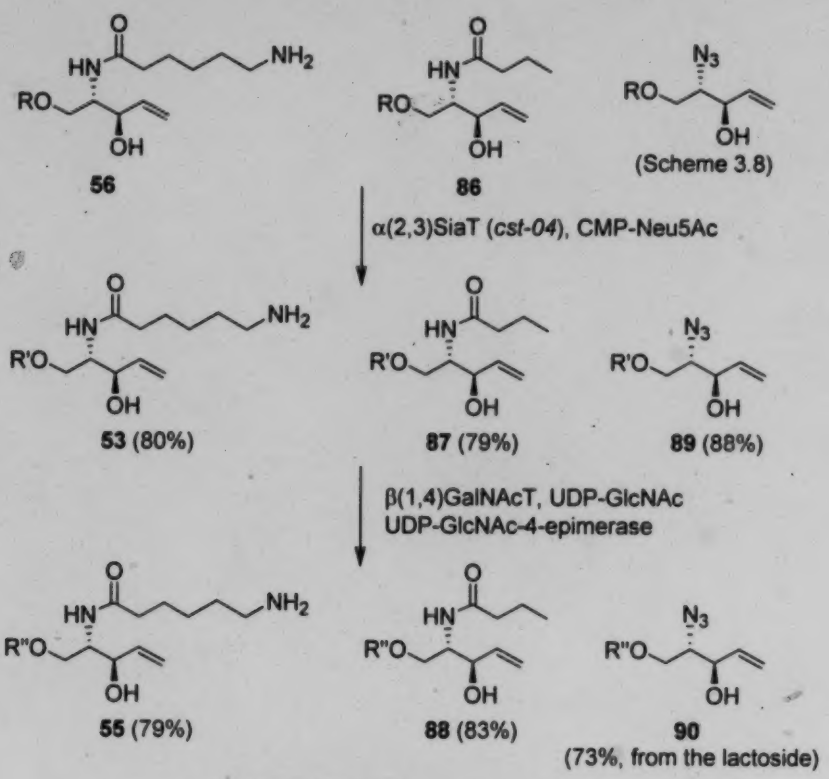
The lactosides **56** and **86** served as acceptors for an α(2,3) sialyltransferase from *C. jejuni*,[217] and the trisaccharide products **53** and **87** were obtained in moderate yields after multiple purifications on C_{18} SepPak cartridges and by reverse phase HPLC (Scheme 3.9). The amino terminated glycosides were isolable by size exclusion chromatography and occasionally required further purification by HPLC. Addition of an *N*-acetylgalactosamine residue via the action of β(1,4) *N*-acetylgalactosaminyltransferase from *C.jejuni* furnished the corresponding tetrasaccharides **55** and **88**. These reactions were conducted on scales ranging from 2-50 mg of starting material. Size exclusion chromatography ultimately proved to be most efficient for the isolation of both tri- and tetra-saccharides. With this knowledge we were able to successfully revisit glycosylation of the unprotected lactosyl azidosphingosine (Scheme 3.9),[166] which could be elaborated to give the larger oligomers **89** and **90**. By leaving the reduction-acylation step to the end of the synthetic route, the flexibility of this synthesis and the utility of the truncated azidosphingosine approach was significantly enhanced.



Scheme 3.8. *Synthesis of acceptor substrates for α(2,3) sialyltransferase.*

Enzymatic sialylation of a variety of galactosyl acceptors was explored (Scheme 3.10). 8-Methoxycarbonyloctyl β-D-galactopyranose served as an acceptor for the α(2,3) sialyltransferase, but concomitant hydrolysis of the methyl ester resulted in the formation of a 1:1 mixture of products. This hydrolysis was attributed to residual esterase activity in the crude enzyme preparation. The alkyl amine terminated sialyl galactose disaccharide **92** was obtained via enzymatic sialylation of 6-azidohexyl β-D-galactopyranoside[218] in 94% yield followed by reduction of the azide **91** with triphenylphosphine in 78% yield. The allyl glycoside **59** was also prepared enzymatically in reasonable yield. Deprotection of the *S*-linked allyl disaccharide **32** by acid catalyzed hydrolysis of the benzylidene acetal followed by Zemplén transesterification and saponification yielded the sialyl galactoside **58**. Interestingly, approximately 10% of the 2-keto propyl glycoside **94**, formed by an unknown

mechanism, was isolated following treatment of **32** with aqueous acetic acid. Efforts to elaborate either of the Neu5Acα(2→3)Gal disaccharides **58** or **59** were unsuccessful.

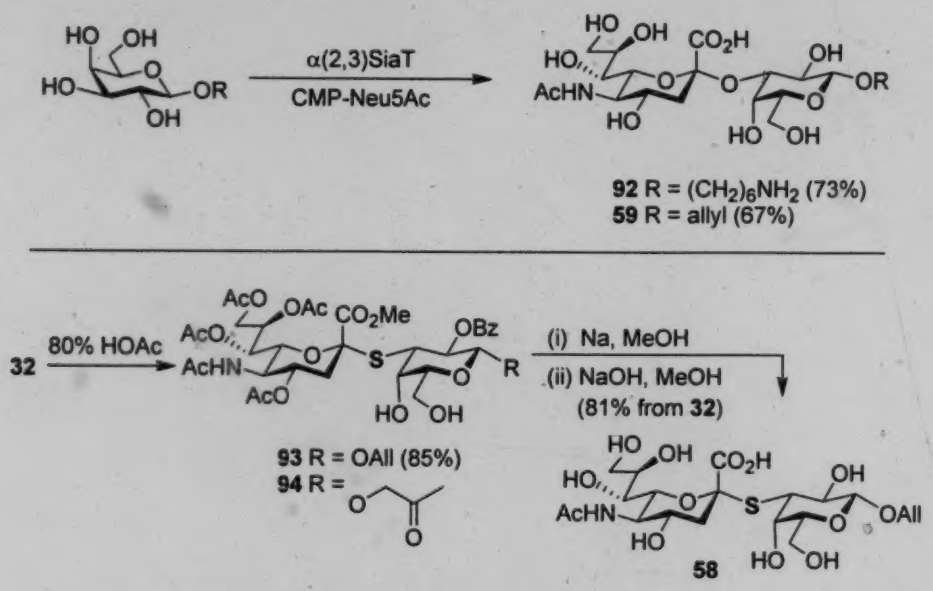


Scheme 3.9. *Enzymatic synthesis of GM_3 and GM_2 oligosaccharides.*

3.5 Thioglycosylceramides

Several groups have proposed *S*-linked glycosyl ceramides as hydrolytically stable components of ganglioside analogues.[219,220] We sought to develop methods by which a thiol containing azidosphingosine homologue could be synthesized and subsequently coupled with oligosaccharides. Our initial approach involved reaction of the azido diol[166] (Scheme 3.11) with thiolacetic acid under Mitsunobu conditions, which

afforded the thioester **95** in 77% yield. In a similar reaction sequence, a thioester was introduced in high yield via the mesylate **96**.

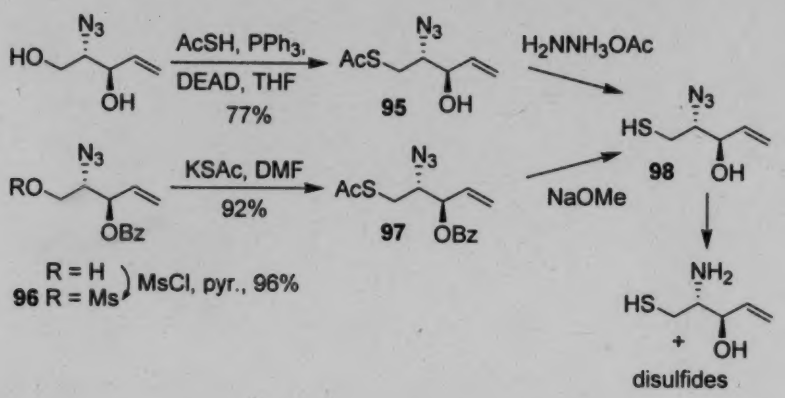


Scheme 3.10. *Chemical and enzymatic synthesis of sialyl galactosides.*

Deprotection of **95** or **97** with hydrazine acetate or sodium methoxide, respectively, afforded the thiol **98**. All ^{1}H NMR, ^{13}C NMR, and IR data, obtained immediately after isolation of the thiol were in accord with the expected structure. However, after a brief storage period the solid was observed to have decomposed to a number of products. HRMS analysis suggested the mixture contained thiol **98**, the related amine, and a mixture of disulfides.

An alternative strategy avoided the potential incompatibility of a thiol and azide. The displacement of a leaving group such as a triflate or mesylate **96** at C-1 of the azidosphingosine unit by a glycosyl thiol(ate) would provide an alternative means of

generating a thioglycosyl ceramide. However, as judged by ^{1}H NMR, a mixture (2:5) of α and β glycosyl ceramides were generated, which proved extremely difficult to separate.



Scheme 3.11. *Synthesis of a 1-thio-azidosphingosine homologue.*

3.6 Conclusions

Combined chemical and enzymatic synthetic techniques have allowed the synthesis of a number of oligosaccharides related in structure to gangliosides. Each of these molecules has been tailored to facilitate evaluation of the sulphur containing GM_3 and GM_2 analogues **52** and **54** as immunogens. The ability of *S*-glycoside **52** to serve as an acceptor for Leloir glycosyltransferases suggests that this molecule does indeed adopt similar conformations to the native trisaccharide, at least in the bound state.

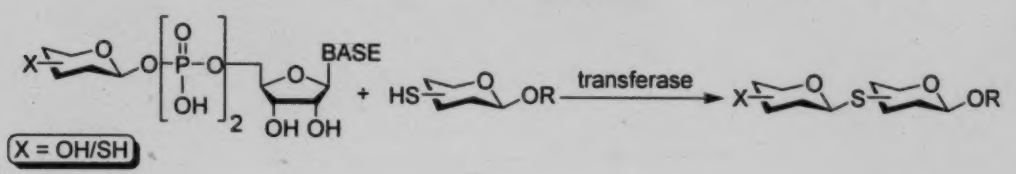
Chapter Four

Glycosyltransferase Catalyzed Synthesis of Thiooligosaccharides

4.1 Introduction

Glycosyltransferases have evolved to become highly efficient catalysts of carbohydrate biosynthesis. Enzymatic synthesis in the laboratory by means of glycosyltransferases has been recognized as an exciting opportunity to minimize laborious protecting group manipulation and difficult separation of diastereomeric products of glycosylation reactions, from the time when their use was pioneered by a number of groups.[221-224] Since then, the production of oligosaccharides with glycosyltransferases has become common in research and industrial settings.[225,226] The pace of development in this field was for some time dictated by progress in the identification, isolation, cloning and expression of the enzymes. However, major advances in molecular biology have removed many of the hurdles, and in excess of thirty glycosyltransferases have now been cloned, while over 7200 sequences have been identified.[227] The glycosyltransferase catalyzed synthesis of oligosaccharide analogues via modified glycosyl donor or acceptor molecules offers a means to access molecules for the study of carbohydrates in a broad range of systems and also the structure, mechanism and other features of the enzyme itself.[204,205,228] With the exception of the enzymes that catalyze the formation of simple thioglycosides in nature, glycosyltransferases have not previously been shown effect the formation of thioglycosidic linkages.

The construction of thioglycosidic linkages with glycosyltransferases by addition of a glycosyl donor to a carbohydrate thiol is readily envisioned (Scheme 4.1). While this strategy would not obviate the often laborious chemical synthesis of the carbohydrate thiol, it offers a potential means to confer to the coupling step the characteristically high yields, regio- and stereo- selectivities associated with glycosyltransferase mediated processes, and might find use in the glycosylation of otherwise unstable substrates. Furthermore, thiol-modified sugar nucleotide donors, if stable and tolerated by the transferase in question, offer a prospective means of simplifying the construction of carbohydrate thiol acceptors.[229-231] The proposed methodology stands apart from reports of the enzymatic modification of thiooligosaccharides,[211] the generation of *O*-glycosidic linkages via glycosylation of thiooligosaccharides by transglycosylases[232] or Leloir glycosyltransferases,[206,207] and the chemoenzymatic synthesis of oligosaccharide analogues containing sulfur in place of the endocyclic oxygen atom.[233] Unsuccessful attempts at enzymatic glycosylation of carbohydrate thiols have been reported,[160] and in one case a carbohydrate thiol was shown to act as an inhibitor of rat liver α(2-6) sialyltransferase (K_i = 3 mM).[234]



Scheme 4.1. *Idealized biocatalytic thiooligosaccharide synthesis using thiol modified donors/acceptors.*

Lemieux introduced the term "key polar group" to describe hydroxyl or other substituent groups that are critical in protein-oligosaccharide recognition.[235] The

evaluation of a number of glycosyltransferases, and synthetic analogues of known acceptor structures that were deoxygenated at the site of glycosylation, revealed that the acceptor hydroxyl did not necessarily participate in key polar contacts.[236] Where recognition of the hydroxyl is not critical, the deoxy analogue is a potential inhibitor of the enzyme. Little is known regarding the capacity of thiols to mimic key polar hydroxyl groups either at or near the site of glycosylation. Although the thiol may be expected to serve as a hydrogen bond donor, its ability to serve as an acceptor should be diminished relative to the hydroxyl group. It must also be noted that in the case of hydroxyl groups oriented about the periphery of the binding site, replacement by a less polar substituent can in fact enhance its affinity for the receptor.[237]

4.2 Attempted glycosyltransferase catalyzed glycosylation of carbohydrate thiols

4.2.1 Transfer to 3'-thiolactose

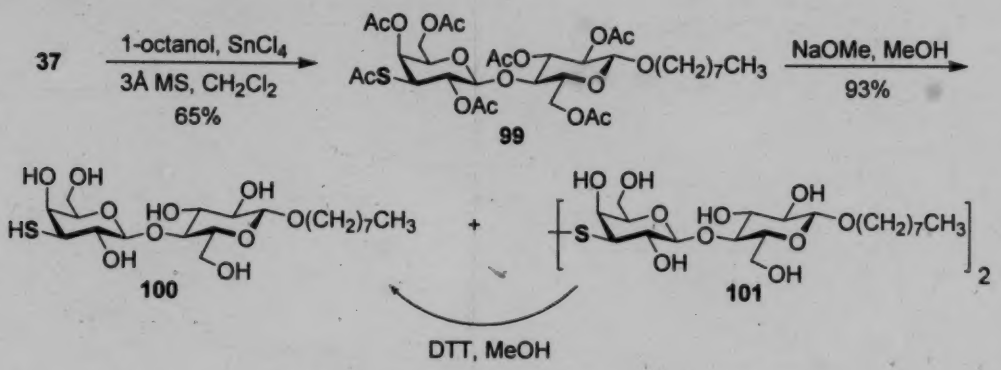
The availability of protected 3'-thiolactose from earlier work prompted consideration of enzymes that catalyze glycosyl transfer to the 3'-position of lactose. α(1,3)-Galactosyl transferase (α(1,3)GalT, EC 2.4.1.151) catalyzes the galactosylation of *N*-acetyllactosamine epitopes on glycolipids and glycoproteins. Lactose and related disaccharides have also been shown to serve as substrates for bovine α(1,3)GalT ($k_{lac/lacNAc}$ = 0.2),[238,239] and this reaction has been conducted on 50g scale using recombinant enzyme.[226] The product of this reaction, αGal(1→3)Galβ(1→4)Glc binds to Toxin A produced by *Clostridium difficile*, a causative agent of antibiotic associated diarrhoea and pseudomembranous colitis, and has potential therapeutic utility.[233]

To identify glycosylation of modified oligosaccharide acceptors a sensitive method for the detection and quantification of product is required, as acceptor analogues

frequently display rates of transfer that are diminished by two or more orders of magnitude.[204,240] The use of a hydrophobic acceptor aglycon such as an alkyl glycoside in conjunction with ^{3}H-labelled nucleotide sugar donors followed by isolation of product on reverse phase (C_{18}) silica cartridges provides such a means.[241] Octyl and 8-methoxycarbonyloctyl glycosides are commonly utilized for this purpose. The nine carbon chain can be conjugated to protein or other molecules following glycosylation, but residual esterase activity in many enzyme preparations often favours the use of the octyl glycoside.

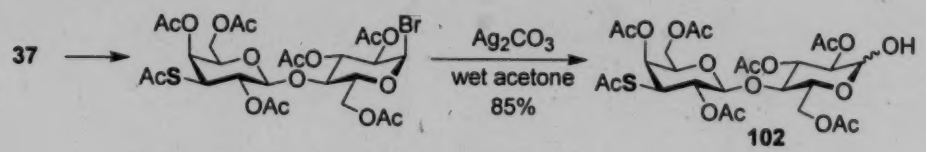
Previous efforts to elaborate 3'-thiolactose octaacetate **37** were frustrated by the similar reactivities of the thioester and the anomeric acetate towards nucleophilic attack. However, transformation of the anomeric ester into a glycoside, either directly or indirectly, would involve cleavage of the C_1-O_1 bond rather than the O_1-C_{ester} bond and thus merited further investigation. Treatment of **37** under standard conditions (hydrogen bromide in acetic acid/dichloromethane) yielded the glycosyl bromide and a substantial amount of side product. Reaction of the crude bromide with 1-octanol in the presence of silver triflate gave a low yield of the target glycoside **99**, and purification proved difficult. Direct glycosidation of 1-octanol in the presence of boron trifluoride diethyl etherate also gave poor results. On the other hand, reaction of the alcohol, tin tetrachloride, and the protected lactose derivative in dichloromethane at 0°C provided the desired lactoside **99** in acceptable yields (Scheme 4.2). Subsequent deprotection under Zemplén conditions afforded the thiol **100**. Rigorous efforts to exclude oxygen from the reaction mixture were required to minimize disulfide formation. A small amount of the thiol was intentionally allowed to form disulfide, yielding **101**. It was also demonstrated

that the thiol could be recovered by treatment with dithiothreitol (DTT) followed by purification on a reverse phase cartridge.



Scheme 4.2. *Synthesis of a 3'-thiolactoside as a potential glycosyltransferase substrate.*

Following this investigation, generation of the glycosyl bromide was revisited. It was found that treatment of **37** with a more dilute solution of hydrogen bromide at 0°C provided the halogenose more cleanly (Scheme 4.3). Hydrolysis of the glycosyl bromide by exposure to silver carbonate in wet acetone gave the hemiacetal **102**.*

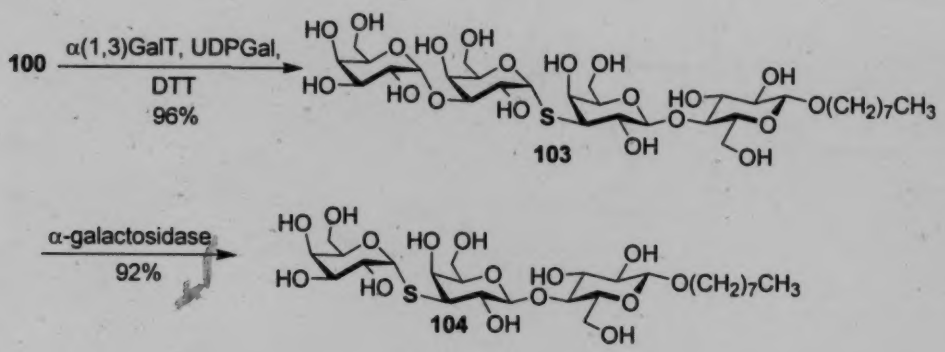


Scheme 4.3. *Synthesis of hemiacetal **102** via hydrolysis of the glycosyl bromide.*

Concerns regarding disulfide formation prompted inclusion of DTT in the enzymatic reaction. Unexpectedly, incubation of a mixture of the thiol and its disulfide (~3:1) with UDP-Gal and unit quantities of recombinant bovine α(1,3)GalT in the presence of DTT afforded the thio-linked *tetrasaccharide* **103** in 92% yield. Thus,

* The hemiacetal could be converted to the trichloroacetimidate and coupled with various alcohols in reasonable yields.

following the initial transfer of a galactosyl residue to the thiol acceptor, a second glycosyl transfer occurred. The unusually large $^{3}J_{H1,H2}$ value (5.6 Hz) observed for the non-terminal α-galactoside H-1 in the ^{1}H NMR spectrum is explicable in light of the atomic substitution at this site.[242-244] Glycosylation of α-linked galactosides by α(1,3)GalT is not totally without precedent, as low levels of product formation have been detected when α-methyl galactose was tested as a substrate.[239,245] The high yields observed were testament to the efficacy of the reducing agent in maintaining free thiol in the reaction mixture. Treatment of 8-methoxycarbonyloctyl β-lactoside under the same conditions yielded the trisaccharide,[246] and a second compound which was identified as the tetrasaccharide by analysis of the high resolution mass and ^{1}H NMR spectra of the mixture.

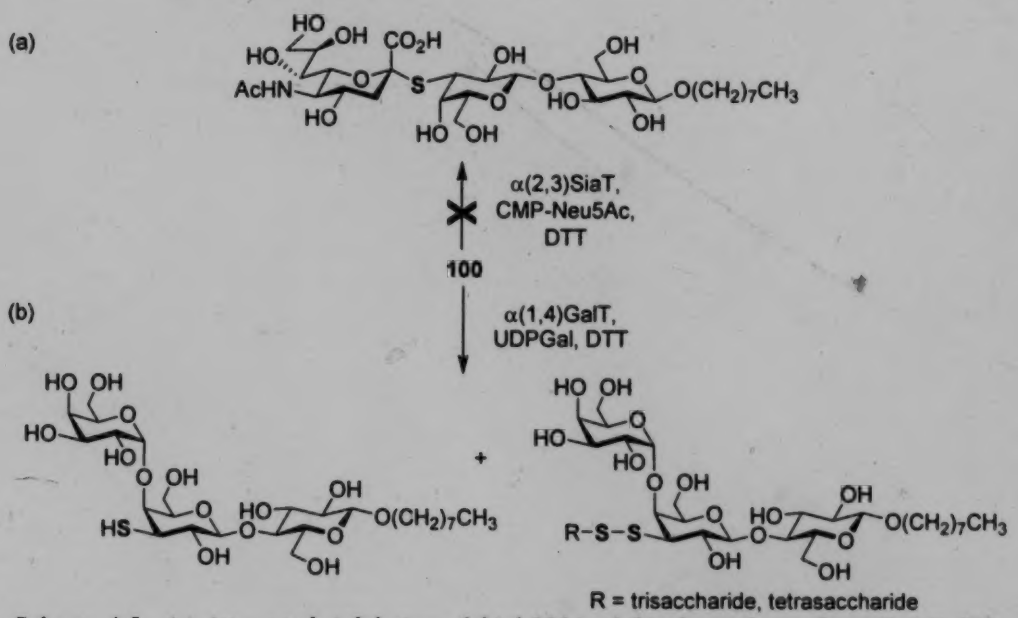


Scheme 4.4. *Sequential transfer of α-linked galactosyl residues to thiol **100**. The O-glycoside could be selectively hydrolysed by α-galactosidase.*

The marked difference in stability between *O*-glycosyl and *S*-glycosyl linkage could be exploited, and the desired trisaccharide **104** was obtained in near quantitative yield following treatment of **103** with an α-galactosidase from green coffee bean [E.C. 3.2.1.22] (Scheme 4.4). This synthetic sidetrack inadvertently reinforced the rationale

for the synthesis of thiooligosaccharides.

The activity of the thiol and the intermediate thiotrisaccharide as acceptors for bovine α(1,3)GalT were determined by a standard radiochemical assay which measured incorporation of ^{3}H-galactose from a commercially available donor.[241]* A substantial decrease in the rate of transfer to the thiol **100** was observed relative to β-octyl lactose. Kinetic analysis showed that the V_{max} for **100** was 0.2% of that of the alcohol; there was also an effect on K_M which increased from 5 mM to 15 mM. Trisaccharide **104** is a better substrate than the thiol with a V_{max} 17% of the parent lactoside and a K_M of 7 mM, accounting for the preferential formation of tetrasaccharide **103**. In light of the similarity of the K_M values for acceptor **100** and β-octyl lactose, and the substantially diminished rate of transfer to the thiol, it seems evident that the apparent nucleophilicity of the thiol is greatly subdued in the enzyme active site.



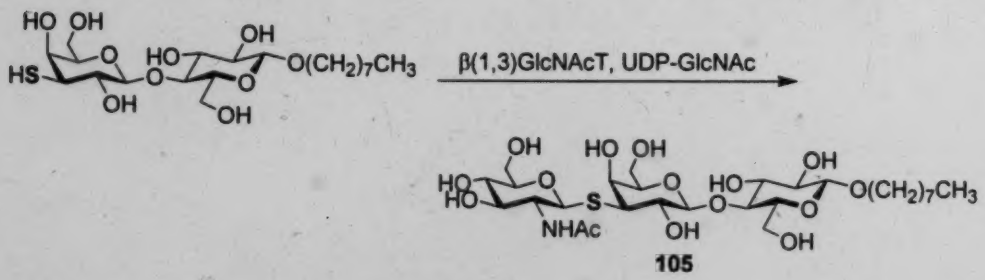
Scheme 4.5. *(a) Attempted sialylation of thiol **100** and (b) glycosylation by α(1,4)GalT.*

* All kinetic measurements were made by Dr. Adam Szpacenko

The lactoside **100** was a potential acceptor for other available glycosyltransferases. The acceptor was evaluated by both radiochemical assay and small scale preparative reaction with a fusion of the CMP-Neu5Ac synthetase from *N. meningitidis* with α(2,3)SiaT from *C.jejuni* OH4384,[217] but no product was observed (Scheme 4.5(a)). This bacterial sialyltransferase has been shown to retain its activity in the presence of reducing agents, unlike the mammalian sialyltransferases.[247] Out of curiosity, thiol **100** was treated with a recombinant α(1,4) galactosyltransferase (α(1,4)GalT) from *N.meningitidis*.[248] Analysis of the ^{1}H NMR and mass spectra revealed a mixture of three products that appeared to include the target trisaccharide thiol and its disulfide, as well as a mixed disulfide formed by reaction of the trisaccharide with a tetrasaccharide (Scheme 4.5(b)). While the exact nature of these products remains uncertain, it is clear that the acceptor is glycosylated by the action of α(1,4)GalT at a site other than the thiol.

β-Lactosides are known acceptors for transfer of *N*-acetylglucosamine by β(1,3)*N*-acetylglucosaminyl transferase (β(1,3)GlcNAcT) from *Neisseria meningitidis*, which has been cloned.[249] The capacity of this enzyme to effect glycosylation of the 3'-thiolactoside **100** was examined by radiochemical assay. The V_{max} value for glycosylation of the thiol to form **105** was found to be 0.5% that of β-octyl lactose. Unfortunately, the combined instability of the enzyme preparation and the poor acceptor characteristics of the thiol prevented successful scale up of the reaction shown in Scheme 4.6. High resolution mass spectrometry of the partially purified reaction mixture identified a product of the correct molecular weight (*m/z* 696.2870), lending further

credence to the suggestion that glycosylation of the thiol occurred. In combination the radiochemical and mass spectrometry results do not preclude the formation of a regioisomeric *O*-glycoside. However, the absence in the mass spectrum of a peak corresponding to a disulfide (5 or 6 saccharide residues) suggests that this is not the case.



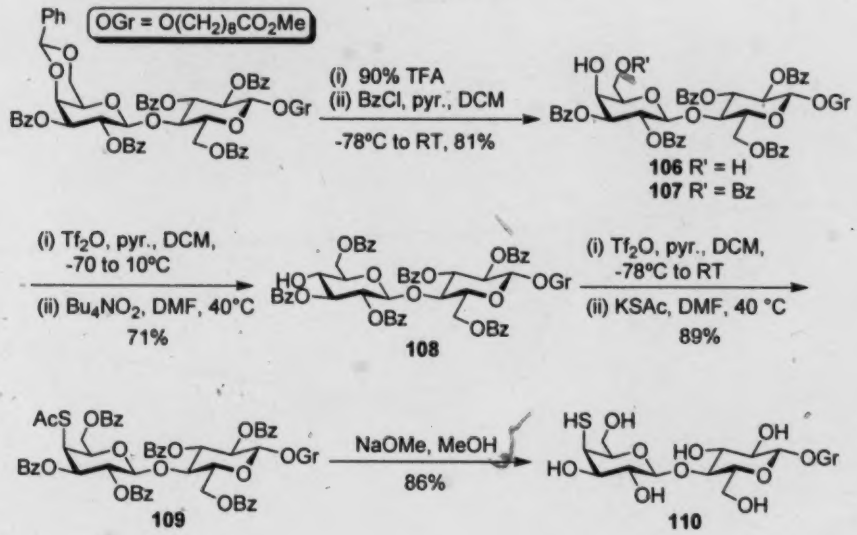
Scheme 4.6. *Glycosylation of thiol* ***100*** *by β(1,3)GlcNAcT.*

4.2.2 Synthesis and evaluation of 4'-thiolactosides as glycosyltransferase substrates.

The evaluation of other glycosyltransferases required access to different carbohydrate thiols. It was envisaged that 4'-thiolactose could be obtained by epimerization of a suitably protected cellobiose derivative, or alternatively by a double inversion sequence at C-4' of lactose. This thiol was viewed as a potential substrate for α(1,4)GalT.[248]

Owing to its availability in multigram quantities, 8-methoxycarbonyloctyl β-lactoside was selected as a starting material. Reaction of the lactoside under previously reported conditions afforded 8-methoxycarbonyloctyl (4,6-*O*-benzylidene-2,3-di-*O*-benzoyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside.[225] Hydrolysis of the acetal (Scheme 4.7) followed by selective benzoylation of the primary alcohol gave the 4'-OH lactoside **107** in 81% yield over two steps. Activation of the hydroxyl group as its trifluoromethanesulfonates ester allowed inversion of the configuration of C-

4'. A second triflation-inversion sequence yielded the protected 4'-thiolactoside **109**. Deprotection in deoxygenated solvent afforded the potential enzyme substrate **110** in good yield. The tendency of this molecule to undergo disulfide formation appeared considerably lower than the regioisomer **100**. Attempted enzymatic galactosylation of thiol **110** failed. No ^{3}H incorporation was detected by standard radiochemical assay and no product was evident by mass spectrometry.



Scheme 4.7. *Preparation of 4'-thiolactoside **110**.*

A GM_2 analogue in which the *N*-acetylgalactosamine residue was linked to the lactosyl core via a thioglycosidic linkage would likely be of utility in the study of the β-hexosaminidase A, a glycoside hydrolase that catalyzes the removal of this residue in the catabolism of ganglioside glycolipids. To evaluate the potential for enzymatic construction of the thioglycosidic linkage using the available bacterial β(1,4)GalNAcT,[213] the trisaccharide Neu5Acα(2→3)-4-thio-Gal-β(1→4)GlcβOR would

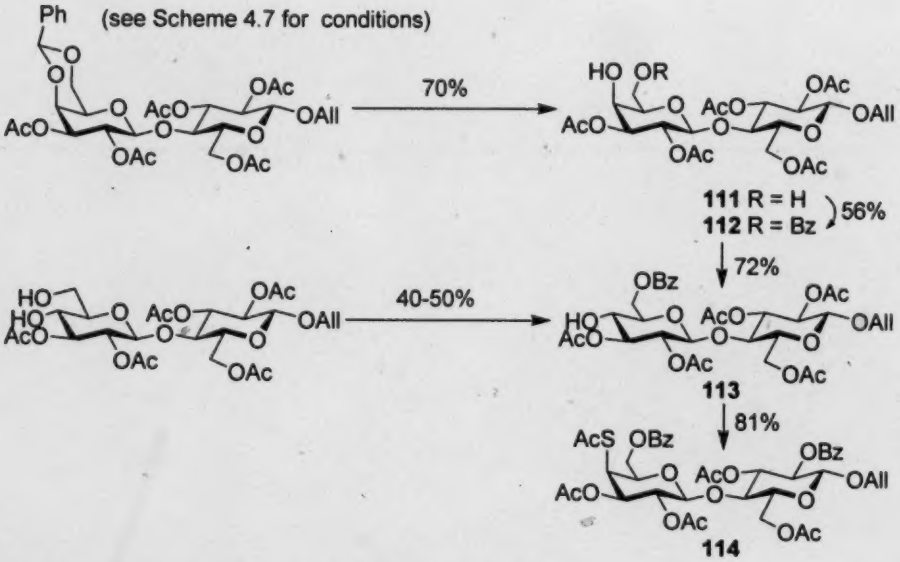
be required as an acceptor. In this case, a smaller aglycon unit would be optimal for crystallographic work involving the desired tetrasaccharide.

In a synthesis paralleling that outlined in Scheme 4.7, an inversion step could be omitted by starting with cellobiose, the 4'-epimer of lactose. Allyl (2,3-di-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranose (Scheme 4.8) was prepared in six steps according to a literature procedure.[250] Selective benzoylation of the primary alcohol was effected under conditions similar to those described above to afford **113**, but yields were variable and low, although the reaction conditions were not optimized. This product was also available from known allyl (4,6-*O*-benzylidene-2,3-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranose.[251] Hydrolysis of the benzylidene acetal gave the diol **111**, which, when treated with benzoyl chloride and pyridine under the same conditions shown in Scheme 4.7, gave **112**, also in moderate yield. These results suggested that the substitution of acetate for benzoate protecting groups was likely the origin of the diminished yields. It is likely that optimization of the reaction conditions might provide higher yields of **113** by either route. Treatment of **113** under standard conditions gave the protected 4'-thiolactoside **114** in 81% yield. 4'-Thiolactoside **110** did not serve as an acceptor for the bacterial α(2,3)SiaT.[217] Protected lactoside **114** may prove a suitable intermediate in a chemical synthesis of the Neu5Acα(2→3)-4-thio-Gal-β(1→4)GlcβOAll trisaccharide.

4.2.3 Synthesis of potential acceptors for the human blood group glycosyltransferases A and B.

The human blood group A and B determinants are trisaccharide structures. The final biosynthetic step in the synthesis of both the A and B antigens involves addition of an α-linked *N*-acetylgalactosamine or galactose residue by either blood group

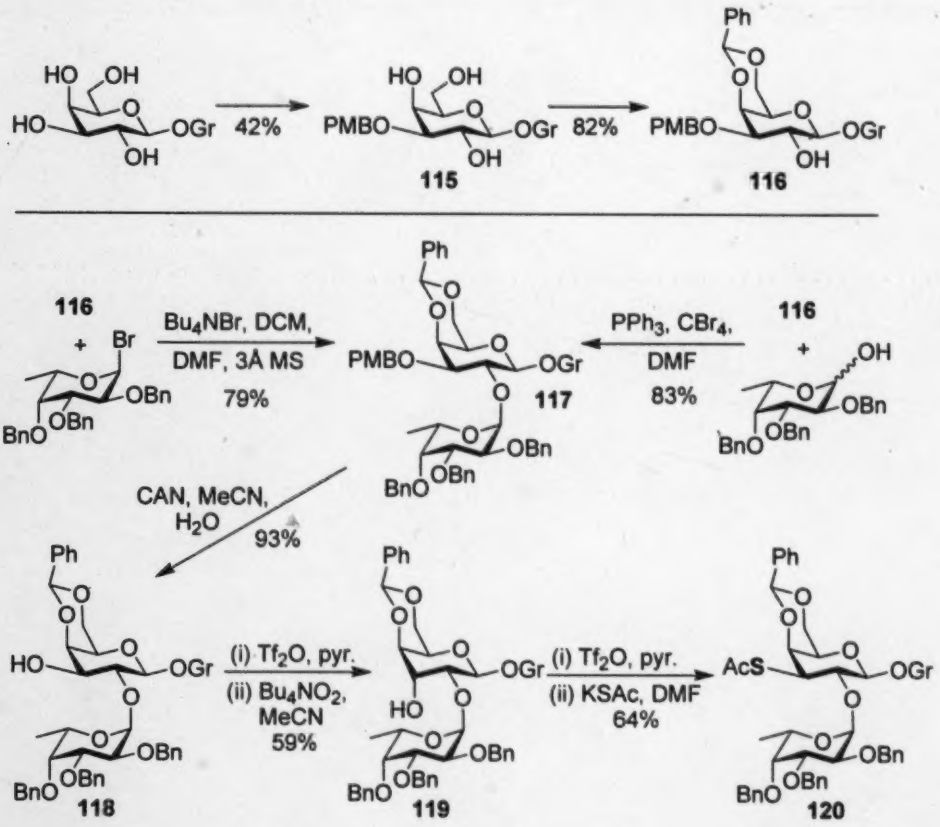
glycosyltransferase A or B (GTA, E.C. 2.4.1.40 and GTB, E.C. 2.4.1.37).[252] Acceptor structures for these enzymes terminate in the H-disaccharide (Fucα(1→2)GalβOR). The site of glycosyl transfer by these enzymes, *O*-3 of the galactose residue, is not a key polar group. As a result, deoxygenated analogues bind to and inhibit these enzymes (K_i = 14-68μM).[253] A 3-thio analogue of the H-disaccharide might serve either as an acceptor or as an inhibitor.



Scheme 4.8. *Synthesis of protected 4'-thiolactoside* ***114***.

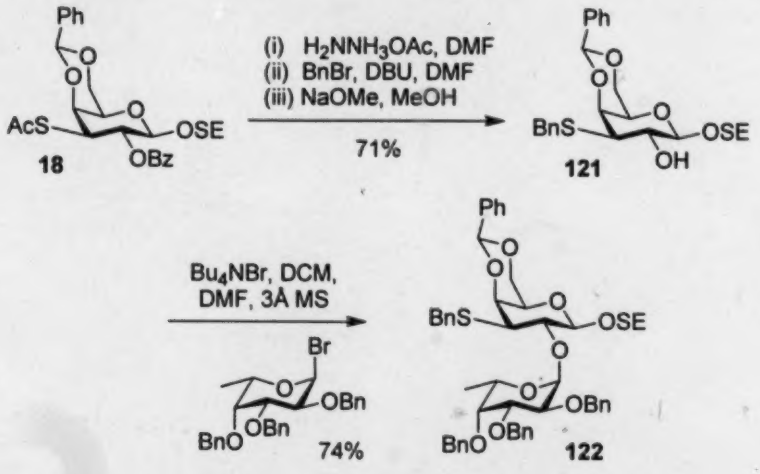
8-Methoxycarbonyloctyl β-galactoside was selected as a starting point. Synthesis of the galactose acceptor **116** was conducted (Scheme 4.9) as described for the 2-(trimethylsilyl)ethyl glycoside **11** (Scheme 2.8). Fucosylation of **116** using either Lemieux's halide ion glycosylation methodology,[254] or a more recently reported dehydrative glycosylation protocol gave essentially the same result.[255] Removal of the *p*-methoxybenzyl group with ceric ammonium nitrate followed by inversion of

stereochemistry at C-3 yielded the guloside **119**. A second inversion sequence allowed installation of the sulfur atom to give **120**. Yields in this reaction sequence are not optimized. Attempts to deprotect the disaccharide by dissolving metal reduction led to mixtures of the 3-deoxy disaccharide and the target thiol (~3:1), which could not be separated. The presence of the target molecule was suggested by analysis of ^{1}NMR spectra of the mixture and by high resolution mass spectrometry (*m/z* 521.2028). Despite the known inhibitory effects of the 3-deoxy disaccharide, this mixture was incubated with GTB (30 U/mL) with the expectation that a radiochemical assay could detect any product formation, but no ^{3}H incorporation from labelled UDP-galactose was observed.



Scheme 4.9. *Synthesis of a 3-S-acetyl protected H-disaccharide analogue.*

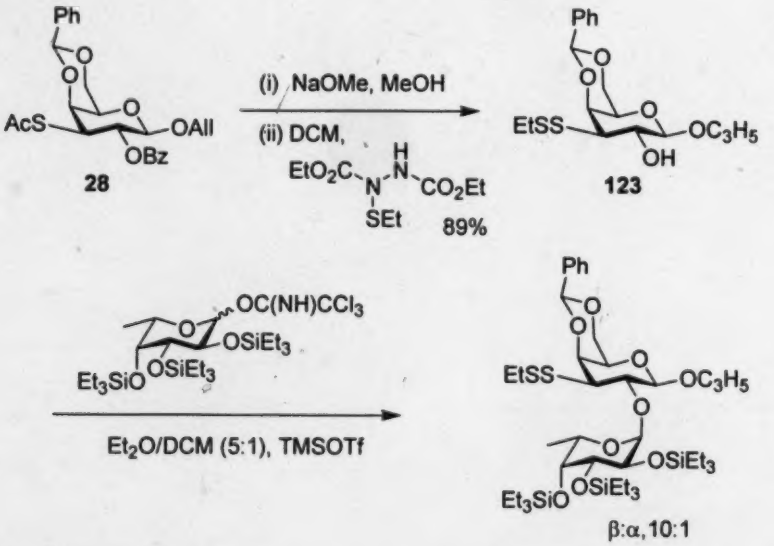
Exchange of the thioester for a benzyl thioether was explored as a means to simplify deprotection. The 2-(trimethylsilyl)ethyl 3-thio galactoside **18** was converted to the 3-*S*-benzyl derivative by selective removal of the thioester followed by treatment with benzyl bromide in the presence of DBU. Treatment of the crude product with sodium methoxide afforded the acceptor **121** (Scheme 4.10). Fucosylation using the halide ion method yielded the disaccharide **122**. Unfortunately, deprotection under dissolving metal conditions gave the 3-deoxy disaccharide and no thiol was detected.



Scheme 4.10. *Synthesis of a 3-S-benzyl protected H-disaccharide analogue.*

A triethylsilyl protected fucosyl trichloroacetimidate has been employed for the α-selective fucosylation of a substrate that was unstable under conditions required for removal of benzyl ethers.[256] If successful, glycosylation of a suitably protected 3-thiogalactoside such as **123** with this donor would afford a product which could be deprotected under relatively mild conditions. The mixed disulfide was available in good yield from allyl glycoside **28** (Scheme 4.11). This strategy is particularly attractive in that the disulfide has the potential to be removed in situ during the course of the

enzymatic reaction if conditions for removal of the other protecting groups are selected with care. Unfortunately, reaction with the trichloroacetimidate in a mixture of diethyl ether and dichloromethane (required for solubility) yielded a 10:1 mixture of the β and α glycosides, as determined by analysis of the crude mixture by ^{1}H NMR (δ 5.63, $J_{1,2\alpha}$ = 3 Hz; δ 4.42, $J_{1,2\beta}$ = 8 Hz). Previously, exclusive α-selectivity was obtained in ether, after optimization of promoter and temperature. Further work could lead to enhanced selectivity in this case. Alternatively, a donor bearing a non-participating group such as a *p*-methoxybenzyl ether at the 2-position might be employed in a successful synthesis of this disaccharide.



Scheme 4.11. *Attempted glycosylation with a triethylsilyl protected fucose donor.*

4.3 Conclusions

It is clear that glycosyltransferases have the potential to catalyze thiooligosaccharide synthesis in some cases. On the other hand, substitution of sulfur for oxygen clearly abrogates the catalytic activity of some enzymes. Further investigation of

the ability of glycosyltransferases to catalyze glycosyl bond formation is merited, and it is likely that some additional synthetically useful processes might be developed from this work. Even among the systems examined thus far, several lines of investigation remain open. Successful synthesis of a 3-thio analogue of the H-disaccharide could yield potentially useful probes GTA and GTB. Additional insight into the nature of specific acceptor-enzyme interactions could also be gleaned by use of the lactose-thiols as substrates for a number of enzymes that glycosylate this disaccharide.

Chapter Five

Immunochemistry of *S*-Linked Oligosaccharides

5.1 Introduction

Vaccines represent a highly effective, cost efficient public health measure in the prevention of infectious disease. These prophylactic vaccines are distinct from those proposed to fight cancer, which are designed to be therapeutic in nature and would likely find use in a multi-therapy approach. An effective vaccine directed against carbohydrate tumor associated antigens would be required to stimulate the production of antibodies to clear circulating tumor cells and thus inhibit metastasis. Furthermore, these antibodies should be specific for the carbohydrate antigen, recognize the target in the context of the tumor cell surface, and demonstrate a high affinity for the glycolipids.

The use of *S*-glycosides in conjugate vaccine preparations has the potential to overcome the low levels of immune response often observed against naturally occurring glycolipids. Either the expected enhancement of the *in vivo* stability of these antigens, their non-self character, or both of these features could result in a strong antibody response to *S*-glycosides. Correlations between extended bioavailability and enhanced immunogenicity have been drawn previously for non-carbohydrate antigens.[257,258] The utility of such an approach would not be limited to the treatment of cancer. Thioglycoside analogues of other disease associated carbohydrates, particularly those susceptible to extracellular degradation by glycosyl hydrolases, might also act as improved antigens. Although the potential utility of metabolically stable oligosaccharides as components of glycoconjugate vaccines has been the subject of much

speculation in recent years,[53,54,56] a report of their *in vivo* evaluation has not yet appeared. Recently, the immunochemistry of *S*-linked analogues of two distinct microbial antigens has been evaluated in the Bundle group.[259] To conduct these experiments, glycoconjugates incorporating *S*-glycosides were required. A preliminary experiment to test this hypothesis might involve immunization of experimental animals with a conjugate vaccine containing *S*-glycosides. Following exsanguination, animal sera would be screened for (i) an antigen specific immune response, (ii) a class switch from IgM to IgG, normally suggestive of the development of immunological memory, (iii) cross-reactivity of antibodies with *O*-glycosides, and (iv) cross-reactivity of antibodies with *O*-gangliosides in the context of a malignant cell. Each of these steps would provide information regarding the potential of this hypothesis. Since the success of this concept is dependent on the conformational similarities between *O*- and *S*-glycosides, measured in terms of cross-reactivity of antibodies with heterosubstituted carbohydrate epitopes, it would also be of interest to immunize with *O*-linked gangliosides and to evaluate the ability of any antibodies produced to recognize *S*-glycosides. This approach would allow comparison of the relative immunogenicity of *O*- and *S*-glycosides provided that the glycoconjugates were of similar composition. Although conducted in the absence of cancer, these experiments could provide an indication of the viability of this strategy and suggest future directions in this field of research.

5.2 Characteristics of the antibody response

The magnitude, specificity, isotype and affinity of immunoglobulin produced must each be considered in the evaluation of an antibody response. The initial molecular

recognition event in the humoral immune response involves binding of antigen to an immunoglobulin receptor at the surface of a naïve B-cell. Binding may stimulate cell proliferation and ultimately the secretion of antibody and generation of immunological memory. The existence in individual mammals of billions of unique B-cells represents an essentially infinite antibody repertoire, each distinguished from all others by the specificity of the antigen receptor, and allows for the generation of a distinct antibody against virtually any foreign molecule. This specificity does not preclude the development of multiple antibodies to a single antigen after interaction of different B-cells with a particular epitope. For example, a glycoconjugate vaccine is likely to stimulate proliferation of B-lymphocytes that secrete antibodies not only against different carbohydrate epitopes of the same oligosaccharide, but also against the surface of the carrier protein and potentially any intermediate linker. As a result, sera obtained after exposure of an animal to a foreign substance is heterogeneous with respect to its immunoglobulin composition. In addition to the production of a variety of antibody paratopes, a number of immunoglobulin isotypes are generated depending on the nature of the antigen and the route by which it is administered, creating an additional layer of complexity in polyclonal sera.

The isotypic variation of an antibody response determines not only their temporal and spatial distribution, but also the ultimate physiological ramifications of the antibodies. The IgM isotype is expressed in monovalent form as a receptor on the surface of B-cells, and is normally the first antibody secreted in response to an immunogen. IgM secreted by plasma B-cells is pentameric and thus the low binding affinity normally associated with this subclass is to some extent compensated for by the

high overall avidity of the pentamer for a complex antigen with repeating epitopes. When the immunogen persists or the animal is subsequently re-exposed to the antigenic stimulus, other classes of antibody may increase in concentration. The IgG isotype is a soluble monomer that normally increases in concentration with a concomitant decrease in IgM levels, and is typically longer lived than IgM in serum. Upon re-exposure to antigen, similar levels of IgM redevelop and are then replaced with more rapid and more intense IgG secretion owing to immunological memory in what is known as the anamnestic response. The extent and rate at which IgM is replaced by IgG is dependent on a variety of factors including the type of antigen, dosage, and route of administration. IgG generally displays a higher affinity for antigen than IgM, having undergone a series of affinity maturation steps via somatic mutation of the amino acid residues in the antigen combining sites. Enhanced affinity corresponds to a higher effective concentration of antibody since fewer molecules are required to eliminate the target cell. The IgG subclass(es) produced also dictate to some extent their effector functions. Some IgG antibody subclasses are capable of fixing complement, enabling the destruction of antigen bearing cells by macrophages.

5.3 Immunization: Experimental considerations.

The immunogenicity of synthetic glycoconjugate vaccines in a given animal model is dependent on numerous variables. The properties of the hapten-carrier conjugate, including the nature of the carrier protein, antigen loading, antigen availability (density, clustering and interaction of surface glycans), the method by which antigen and carrier are coupled and of course the nature of the carbohydrate itself are likely the most important considerations. In addition to the design of the glycoconjugate, the timing of

immunizations, dose, immunological adjuvant, site and method of immunization, and animal model employed are significant factors. Each of the listed considerations may influence the magnitude of the antibody response (titre), affinity, isotype and subclass, anamnestic response, and capacity to bind and eliminate the intended target.

Cancer vaccines would ideally target cancerous cells rather than individual molecules. In this way, the unique arrangement of particular oligosaccharides at the tumor cell surface would serve as a recognition element for the immune system, and reactivity with normal tissues would be minimized. The intimate arrangement of TACA's at the surface of malignant cells undoubtedly impacts on the conformation of the exposed carbohydrates, and thus densely glycosylated cells display conformational epitopes in much the same way as folded proteins or polysaccharides. The oligosaccharide portion of a glycoconjugate vaccine should thus mimic where possible the tumor cell surface. Unfortunately, synthetic complexity and a poorly developed understanding of the orientation of glycolipids at the cell surface are limitations that undermine this consideration. Clustering of glycolipids in vaccine formulations has been proposed as a means to mimic the densely functionalized surfaces of tumor cells and cancer associated glycoproteins, but has thus far met with only limited success.[173,260]

The choice of a carrier molecule is dictated by its immunogenicity, toxicity, stability, and the feasibility of its coupling with antigen.[163] The carrier should be foreign to the experimental animal and should not be glycosylated. Ultimately the primary criterion, the ability to confer immunogenicity to the hapten, is of utmost importance. Although the former properties are relatively easy to evaluate in a given organism, systematic study of the immunogenicity of different antigen-carrier conjugates is required for each system

of interest. As a result the carrier, usually a protein, is often selected from among a small group of well studied molecules or perhaps based on results from related studies of unknown relevance. Common carrier proteins used in glycoconjugate vaccine development have included keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA), tetanus toxoid (TT), and Diptheria toxoid (DT). Liposomal and synthetic carriers have also found use. Co-administration of an adjuvant enhances the immunogenicity of the conjugate by rendering it particulate and stimulating macrophages in the early, non-adaptive phase of the immune response. Bacterial lipopolysaccharide in some formulations is recognized by toll like receptors, which promotes the transition from innate to adaptive immune response by ultimately upregulating MHC II synthesis. Many adjuvants also ensure slow release of antigen, thus prolonging its exposure to the immune system.

The choice of conjugation strategy including the nature and length of a spacer molecule can have a large impact on the success of a glycoconjugate vaccine.[41,261,262] It is more common to activate the carbohydrate rather than the carrier for subsequent coupling with the other component. In this way, side reactions that disrupt the coupling step do not result in the incorporation of anomalous features into the immunogen. However, the scarcity of the synthetic carbohydrate is often an incentive to avoid its further synthetic manipulation. A number of reagents are available to activate the protein surface for coupling with thiols and amines, and several pre-activated carrier proteins are commercially available. Frequently when a reactive carbohydrate is coupled to an unactivated protein, the aglycon is first activated as a succinimide ester, maleimide, *O*-acyl urea, acyl azide, diazonium salt or by reaction of a primary amine with diethyl

squarate (3,4-diethoxy-3-cyclobutene-1,2-dione) or other popular commercially available homo- and hetero- bifunctional linkers such as *N*-succinimidyl-4-(*N*-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC).[163] Although couplings with these linkers are often highly efficient, they contain rigid elements which are in some cases themselves the focus of an immune response.[134,261-263] The integrity of the activated oligosaccharide and the glycoconjugate is also a consideration.

The conjugation strategy employed impacts the antigen:carrier ratio, which may in turn affect the immune response to the conjugate. It is generally observed that multiple copies of the oligosaccharide confer greater antigen specific immunogenicity to the neoglycoprotein. However, optimal levels must be determined for each system and higher hapten densities do not necessarily give better results. It is unclear whether increased hapten density may result in deleterious modification of peptide sequences required for the recruitment of T-cell help following antigen internalization and processing.[264,265]

5.4 Synthesis of protein conjugates

KLH is a large, glycosylated, highly immunogenic multi-subunit protein that suffers from stability and solubility problems and exists in several distinct multimeric forms.[163] Although this carrier is preferred by many experimentalists for the preparation of glycoconjugate vaccines,[40] the associated synthetic obstacles and inhomogeneity of conjugate preparations suggest that its use in clinical practice would not be approved. In addition, antibodies to KLH have been shown to cross react with a wide range of carbohydrates, including some gangliosides.[266] TT has previously provided good results when used as a component of glycoconjugate vaccines in mice and rabbits, and

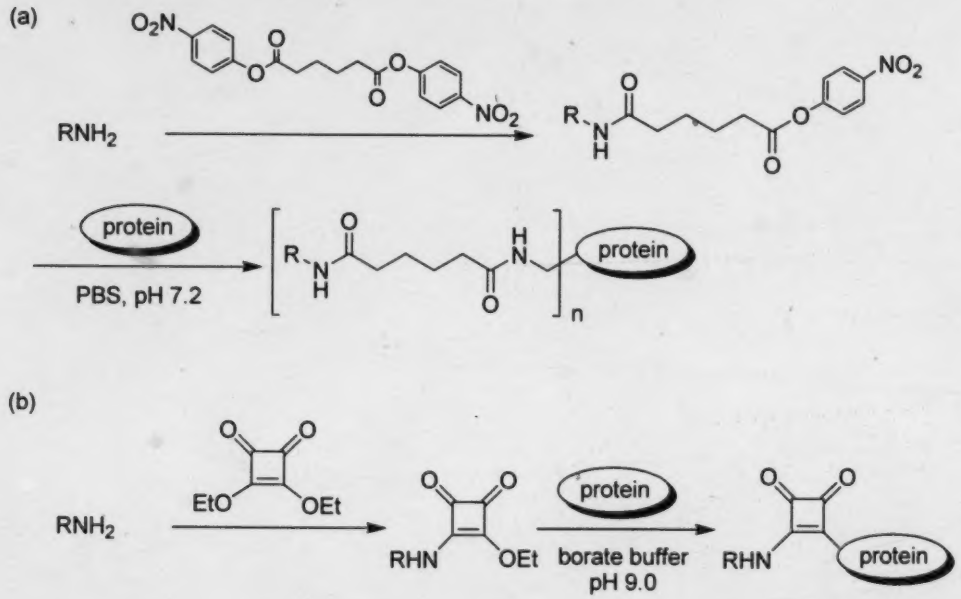
was selected for use in this study. Although this protein also forms multimers they are readily separated by gel filtration chromatography to provide the monomer (MW = 153,000), which may be stored in solution with minimal aggregation allowing for the preparation of well defined conjugates.*

The antibody response to the linker in conjugate vaccines may be minimized by use of a linear aliphatic linker.[263] Homobifunctional reagents derived from adipic acid (1,6-hexanedioic acid) have found use in a variety of fields including in the preparation of glycodendrimers and glycoconjugates.[261,267] In particular the *p*-nitrophenyl and succinimidyl diesters demonstrate sufficient stability and reactivity to allow for activation of an amine terminated oligosaccharide in reasonable yield, and efficient coupling with protein in a subsequent step.[268,269]

Oligosaccharide amines were incorporated into glycoconjugates for immunization or measurement of the antibody response by one of two general methods (Scheme 5.1 (a) and (b)). Reaction of the amines with *p*-nitrophenyl adipate diester yielded the half ester, which could be isolated in reasonable yields following HPLC purification provided that a small amount of acetic acid was included in the eluent (Scheme 5.1(a)). After lyophilization, the activated esters were reacted with tetanus toxoid or BSA in solution (PBS, pH 7.2). Following dialysis, oligosaccharide-TT conjugates were stored frozen at –20 °C, while BSA containing conjugates were freeze-dried. A comparison of these coupling outcomes with other results from the Bundle group suggests that higher incorporation might be realized via use of slightly more basic buffer (pH 7.5). Diethyl squarate was also selected as a cross coupling reagent for preparation of glycoconjugates

* TT was purified by Tomasz Lipinski.

(Scheme 5.1(b)). Following activation of the amine by reaction with diethyl squarate, coupling was done in borate buffer, pH 9.0 (Scheme 5.2).[270] After extensive dialysis against PBS, conjugates were lyophilized and obtained as solids.



Scheme 5.1. *Synthesis of glycoconjugates using (a) adipate and (b) squarate linkers.*

S- and *O*-linked oligosaccharides[1] (**52-55**; Fig. 3.3) for use in immunizations were coupled via a homobifunctional adipate linker to TT. The same molecules and (**56**, **57**, and **92**) were coupled to BSA via a squarate linker for use as screening antigens in ELISA. The use of both heterologous protein (BSA) and linker (squarate) precludes the inclusion of both carrier protein and linker specific antibody in the measurements of immune response to the antigen. A comparison of the recognition of squarate linked thiooligosaccharide-BSA conjugates by immune sera would provide an estimate of the immunogenicity of the adipate linker. The composition of all glycoconjugates used in

[1] *O*-Linked oligosaccharide-TT conjugates were prepared by Sandra Jacques.

this study are shown in Figures 5.1 and 5.2. Average oligosaccharide incorporation was determined by MALDI-TOF mass spectrometry.

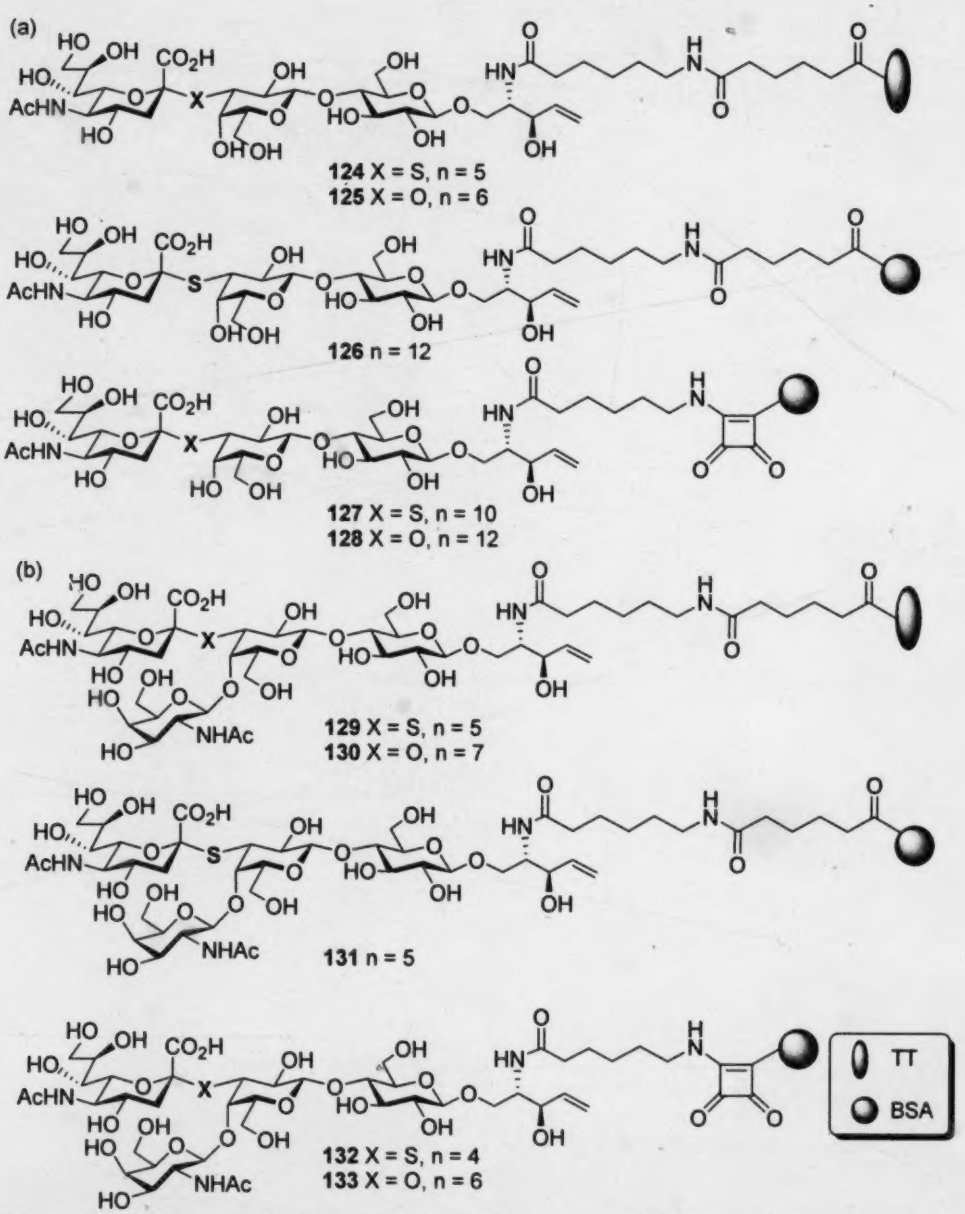
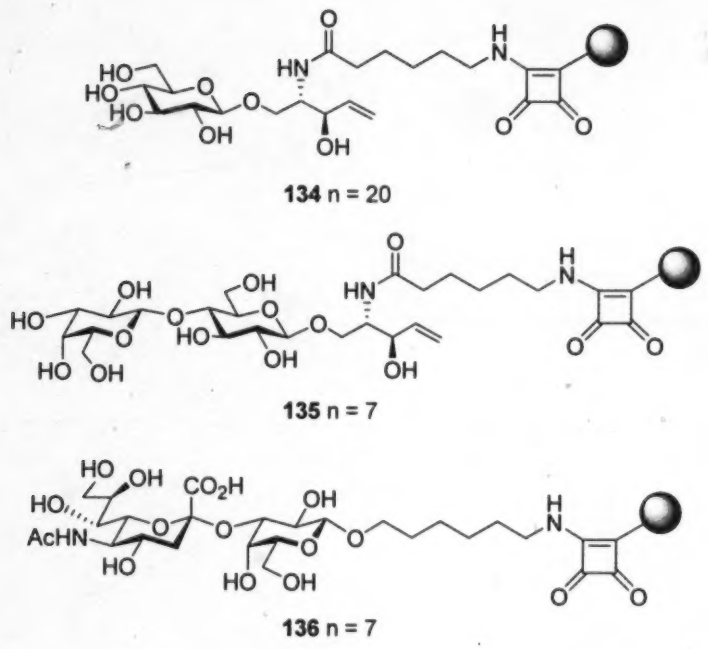


Figure 5.1. *Vaccine and screening glycoconjugates prepared from (a) GM3 and (b) GM2 analogues (n = average saccharide loading).*



134 n = 20

135 n = 7

136 n = 7

Figure 5.2. *Miscellaneous screening glycoconjugates (n = average saccharide loading).*

5.5 Immunization of experimental animals

Groups of five Balb/C mice were immunized with tetanus toxoid conjugates **124**, **125**, **129**, and **130** as described in the experimental section. This inbred strain of mice has previously shown immune responses to TACAs and to tetanus toxoid conjugates and is most often used by immunologists.

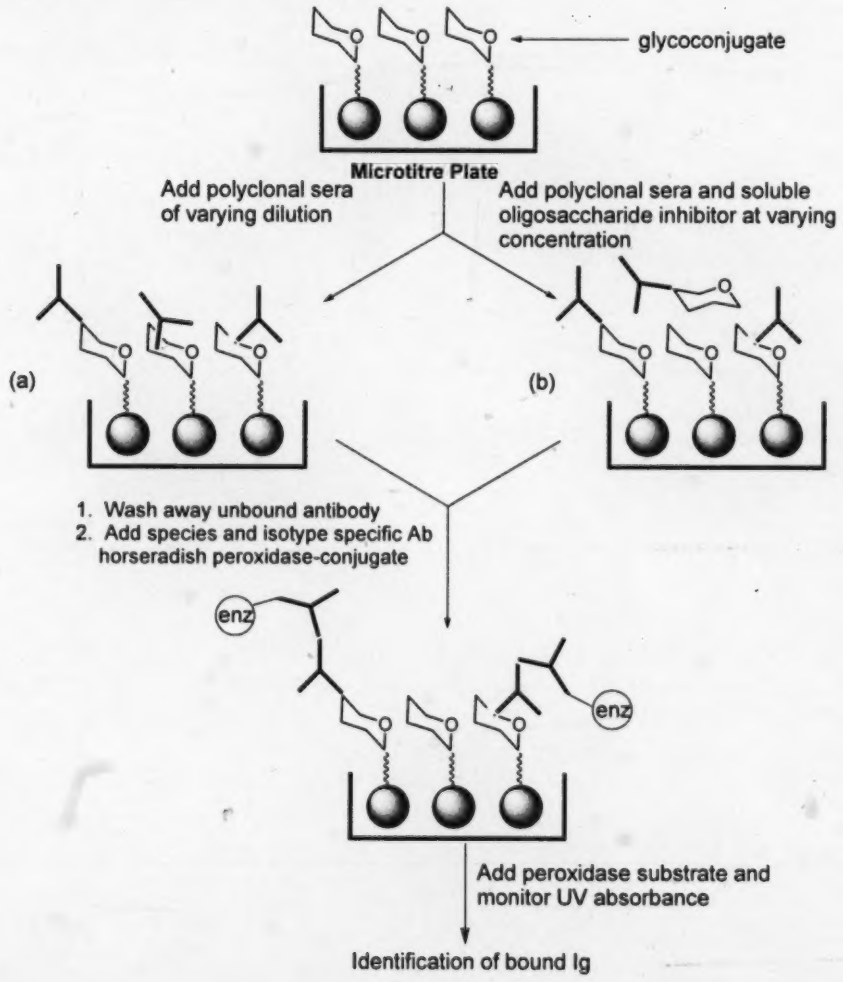
5.5.1 Analysis of antibody levels in immune sera*

Antigen specific antibody response

After three immunizations with one of the thiooligosaccharide-TT conjugates, mice were sacrificed and sera were collected. Antibody levels against a series of

* ELISA work was performed by Mrs. Joanna Sadowska.

glycoconjugates were determined by indirect ELISA. Oligosaccharide-BSA conjugates were immobilized on microtitre plates incubated with sera at various dilutions as outlined in Scheme 5.2. After washing away unbound antibody, a second isotype specific goat anti-mouse antibody-horseradish peroxidase conjugate was added. Relative levels of bound serum antibodies were then detected by addition of an enzyme substrate and colourimetric determination of product formation.


glycoconjugate
Microtitre Plate
Add polyclonal sera of varying dilution
Add polyclonal sera and soluble oligosaccharide inhibitor at varying concentration
(a)
(b)
1. Wash away unbound antibody
2. Add species and isotype specific Ab horseradish peroxidase-conjugate
enz
enz
Add peroxidase substrate and monitor UV absorbance
Identification of bound Ig

Scheme 5.2. *ELISA protocol for (a) determination of titre and (b) IC_{50} values.*

To evaluate the production of antigen specific antibodies to *S*-GM_3, *S*-GM_3-BSA conjugates **126** and **127** were used as screening antigens. The evaluation of both squarate and adipate type linkers allowed for distinction between an immune response to the linker moiety and the saccharide epitope. As shown in Figure 5.3 mice immunized with *S*-GM_3-TT conjugate **124** developed IgG antibodies that recognized either conjugate to a similar degree, suggesting not only that the *S*-glycoside produced an antigen specific immune response, but also that there was no generation of adipate specific antibodies. Of the five experimental animals, four demonstrated a significant antigen specific IgG response to the immunization protocol.

Assay of immune sera to *S*-GM_2-TT conjugate **129** screened against adipate and squarate linked *S*-GM_2-BSA glycoconjugates **131** and **132** is shown in Figure 5.4. The average antigen specific IgG antibody titre against these two glycoconjugates is comparable. Again the linker specific component of the antibody response was negligible. In each case background absorbance readings obtained from pre-immune sera were consistently lower than 0.1 absorbance units.

IgM titres against the squarate linked *S*-glycoside conjugates **127** and **132** were also evaluated for both sets of *S*-glycoside derived sera. In general, the IgM titres were lower than those of IgG, indicating an antigen induced isotype switch. The single mouse deemed unresponsive to *S*-GM_3-TT conjugate **124** in terms of an IgG response actually demonstrated a relatively high IgM titre as might be expected. Similarly the sera with the highest antigen specific binding capacity, in terms of IgG levels, showed the lowest IgM response in each situation, although in the case of *S*-GM_2, differences between the individual sera were minimal. Background absorbance readings in IgM titrations were

considerably higher than those in IgG titration experiments, which is explicable in light of the relaxed binding specificity associated with the former isotype.

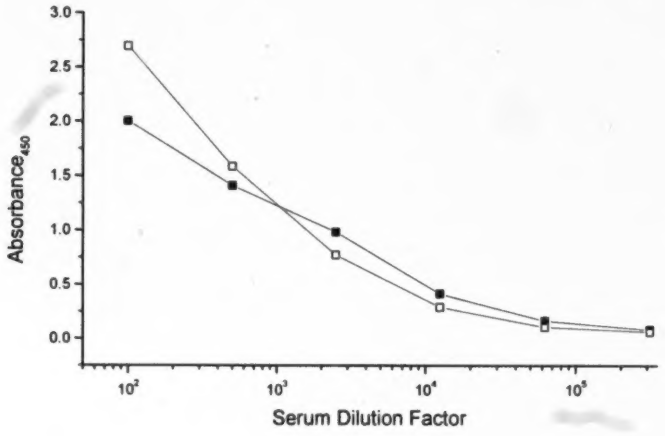


Figure 5.3. *Binding of IgG from polyclonal sera derived by immunization with S-GM₃-TT glycoconjugate* ***124*** *to S-GM₃-BSA glycoconjugates* ***126*** *(■), and* ***127*** *(□). Data are average responses of four mice.*

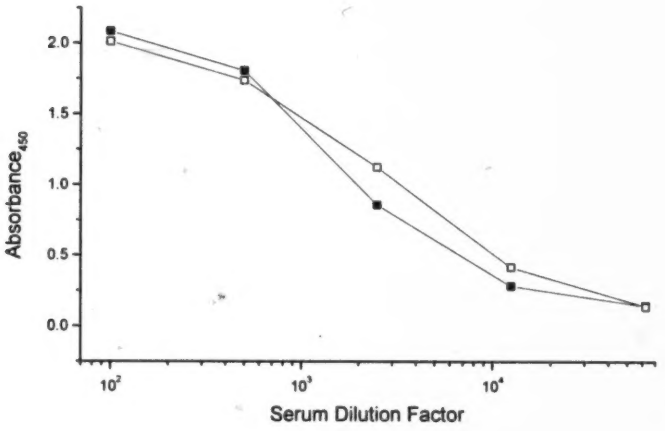


Figure 5.4. *Binding of IgG from polyclonal sera derived by immunization with S-GM₂-TT glycoconjugate* ***129*** *to S-GM₂-BSA glycoconjugates* ***131*** *(■), and and* ***132*** *(□). Data are average responses of five mice.*

Cross reactivity of sera against *S*-glycosides with other oligosaccharides.

***S*-glycosides.**

Sera were evaluated for antibodies to either of the *S*-linked tri- or tetra-saccharide that might recognize the other, related structure. IgG antibodies against *S*-GM_3 **124** were found to recognize *S*-GM_2 (**131** or **132**), but the opposite was not the case (Figure 5.5). Predictably, the more promiscuous IgM isotype was found to bind either oligomer in both cases.

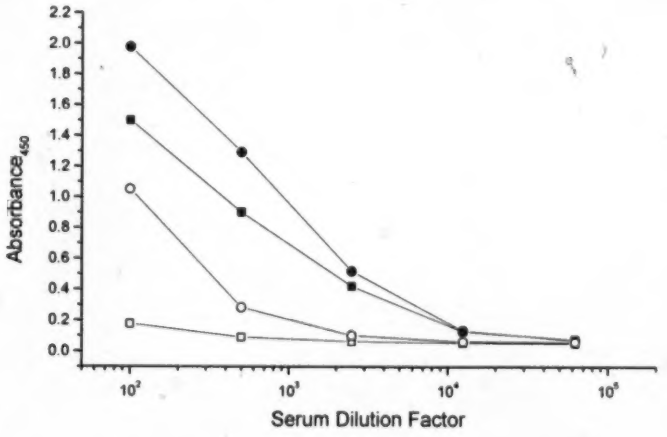


Figure 5.5. *Binding of IgG and IgM from polyclonal sera derived by immunization with conjugate thiotrisaccharide **124** to thiotetrasaccharide **132** (■ IgG; ● IgM), and of sera raised against the thiotetrasaccharide **129** to thiotrisaccharide **127** (□ IgG; ○ IgM). Data are average responses of four mice.*

***O*-Glycosides.**

The propensity of sera derived from thioglycosides to bind *O*-linked oligosaccharides was next evaluated. Cross reactivity would indicate that the bound conformation of the *S*-glycoside resembled that of the *O*-glycoside, or at least that the *O*-glycoside could readily adopt such a conformation.

Among the four sera containing IgG antibodies against the *S*-GM_3 structure, three recognized the *O*-GM_3-BSA-conjugate **128**. Only the serum that had previously shown the weakest recognition of *S*-GM_3 conjugate **127** did not bind to the *O*-glycoside appreciably. A demonstration of the cross reactivity and a comparison of recognition of the two heterosubstituted oligosaccharides is shown in Figure 5.6. It is clear from this data that in this case *S*-linked immunogens can generate significant levels of IgG that bind *O*-glycosides, and that the immunoglobulins have a generally higher affinity for the immunizing structure than the *O*-glycoside.

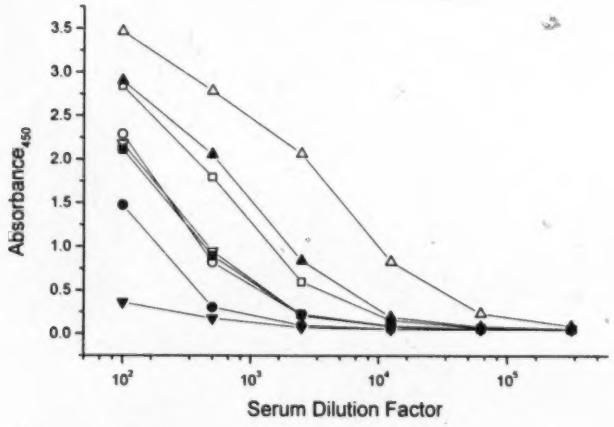


Figure 5.6. *Binding of IgG from polyclonal sera derived by immunization with S-GM_3 conjugate **124** to S-GM_3 **127** (open symbols) and O-GM_3 **128** (closed symbols). Each shape (open or closed) represents serum from the same mouse.*

To better understand the immune response to these antigens and to ascertain the importance of the modified terminal region of the oligosaccharide in antibody mediated recognition, sera to *S*-GM_3-TT conjugate **124** were screened against a series of BSA conjugates (**134-136**) bearing smaller oligosaccharide fragments. Each of the glucosyl, lactosyl and terminal disaccharide fragments was recognized by IgG in sera of the

immunized mice, as shown in Figure 5.7. Absorbance measurements were significant when pre-immune sera were screened against the glucoside and lactoside.

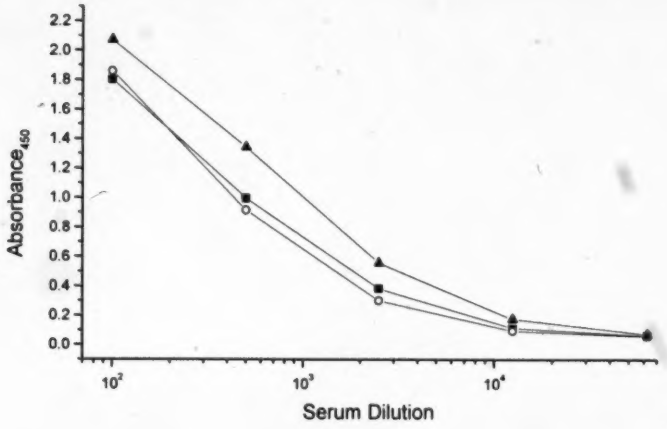


Figure 5.7. *Binding of IgG from sera against S-GM$_3$ glycoconjugate **124** to BSA glycoconjugates **134** (lactose ■), **135** (glucose ○),and **136** (Neu5Ac-Gal▲).*

Inhibition of antibody binding to immobilized glycoconjugates with soluble oligosaccharide ligands provides a more definitive assessment of the specificity of the immunoglobulin. Although the opportunity for non-specific binding of the glycoconjugate by antibody remains possible, this experiment measures the change in binding, which may only be due, in theory, to antibody binding of the monovalent ligand in solution. Inhibition experiments also provide a measure of the avidity of the antibodies via derivation of an average IC_{50} value.

The binding of immune sera against *S*-GM$_3$-TT glycoconjugate **124** to *O*-GM$_3$-BSA conjugate **128** was examined by competitive ELISA (Scheme 5.3b). Inhibition was achieved by addition of varying concentrations of the soluble *O*-GM$_3$ (**87**) and *O*-GM$_2$ (**88**). Both the GM$_2$ and GM$_3$ homologues were found to be good inhibitors of antibody binding (Figure 5.8). Methyl β-glucoside, methyl β-lactoside, and the

Neu5Acα(2→3[*O*/S])Gal disaccharides **58** and **59** demonstrated no inhibitory capacity. Inhibition experiments with the thio-linked immunizing epitope were not conducted owing to the scarcity of this material.

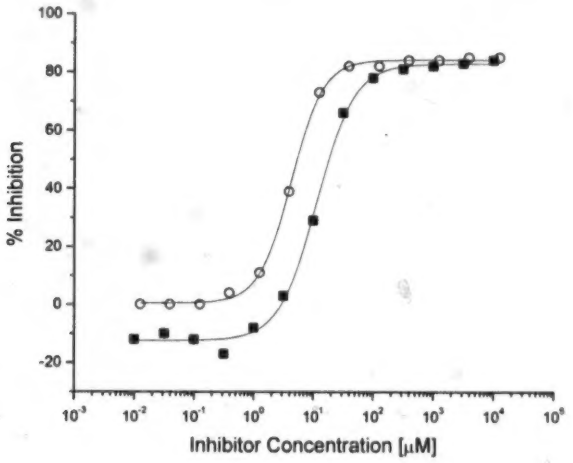


Figure 5.8. *Synthetic oligosaccharides **87** (○) and **88** (■) inhibit binding of IgG from polyclonal sera against S-GM$_3$-TT conjugate **124** to immobilized O-GM$_3$-BSA **128**. IC$_{50}$ values were determined to be 4.2 μM (**87**) and 11.3 μM (**88**).*

Following immunization with *S*-linked GM$_2$-TT conjugate **129** sera were analyzed for cross reactivity with the heterosubstituted glycosides as described above. In this case, the corresponding *O*-glycoside (*O*-GM$_2$-BSA conjugate **130**) was not recognized appreciably by IgM or IgG antibodies in the mouse sera.

Specificity of sera against *O*-GM$_3$ and *O*-GM$_2$-TT conjugates

Tetanus toxoid conjugates of *O*-GM$_3$ and *O*-GM$_2$ were found to induce an antigen specific antibody response in Balb/C mice.* In each case, three of five mice developed high IgG titres against the heterologous protein conjugates, while two mice were judged

* These sera were provided by Sandra Jacques.

not to have responded. Immune sera to the immunizing glycoconjugates **125** and **130** from mice deemed to have produced an antibody response to the immunogen were screened against the thiooligosaccharide-BSA conjugates **127** and **132**. In each case, sera demonstrated recognition of the corresponding *S*-glycoside that was equivalent or superior to the immunizing epitope.

To confirm recognition of the *S*-glycoside, competitive ELISA experiments were conducted with sera against the *O*-GM_3-TT conjugate **125** using *O*- and *S*-glycosides **137**, **89**, and **52** as inhibitors (Figure 5.9).* The *O*- and *S*- glycosides **137** and **52** showed comparable inhibition of IgG binding to *O*-GM_3-BSA (**128**), suggestive of their equivalent recognition by antibodies in the polyclonal sera (Figure 5.10). A second *O*-glycoside (**89**) demonstrated inhibitory power that was at least two orders of magnitude lower than the others, potentially as a consequence of the presence of the polar azido group in the aglycon.

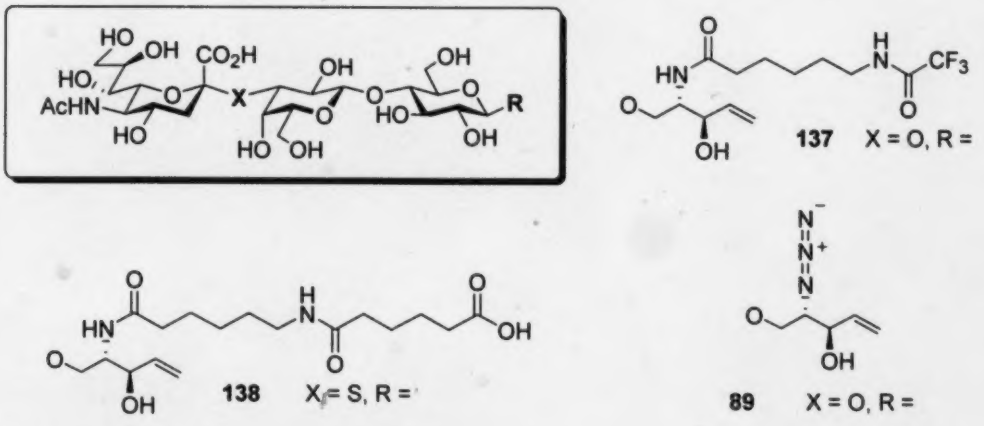


Figure 5.9. *Glycosides used in inhibition experiments.*

* Oligosaccharide **137** was obtained from Sandra Jacques.

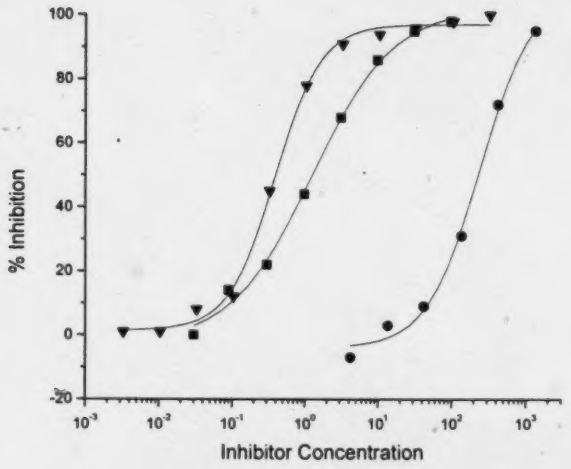


Figure 5.10. *Inhibition of antibody binding to O-GM$_3$-BSA conjugate* ***128*** *by S- and O-linked oligosaccharides* ***137*** *(▼),* ***52****(■), and* ***89*** *(●). IC$_{50}$ values were 0.4, 1.2 and 238 μM, respectively.*

5.5.2 Comparison of the antibody response to *O*- and *S*-ganglioside analogues.

It is difficult to analyze the magnitude of a polyclonal antibody response to different antigens, although some general conclusions can be drawn. It is important to consider differences in the composition of the immunizing epitope as well as the composition of the immunogen itself. The four immunogens evaluated in this study were administered under the same conditions, and utilize the same linker structure and very similar antigen incorporation levels, allowing for evaluation of the relative immune response to each by comparison of their binding to a particular control compound. There are some discrepancies between the antigen loadings of the control compounds. In particular, each of the BSA glycoconjugates bearing *O*-linked tri- and tetrasaccharides (**128** and **133**) contain twice as many oligosaccharide units as are found in the thioglycoside conjugates. Comparison of the titres of the same sera screened against

both heterotypes may be somewhat biased towards the *O*-linked oligosaccharide by a statistical effect. Although likely, this is not necessarily the case as the relative orientations of the oligosaccharide and their ability to affect adhesion and packing on the microtitre plate are unknown.

Titrations of sera obtained from immunization with GM_3 containing glycoforms (**124** and **125**) against both of the GM_3-BSA conjugates **127** and **128** are depicted in Figure 5.11. The antigen specific IgG response was higher against the *S*-glycoside than the *O*-glycoside. Somewhat lower, albeit similar titres were observed when either set of sera were screened against the heterosubstituted oligosaccharide. It is clear that in both cases there is cross reactivity between the two classes of oligosaccharide and the IgG derived from them.

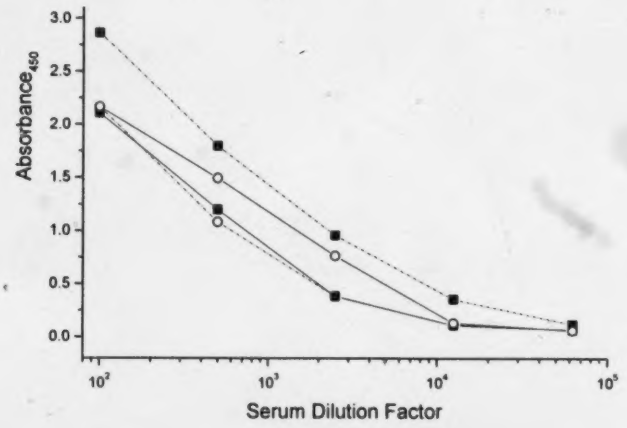


Figure 5.11. *Binding to immobilized O-GM_3-BSA **128** (○) and S-GM_3-BSA **127** (■) by murine IgG generated from immunization of mice with O-GM_3-TT **125** (—) or S-GM_3-TT **124** (---). Data are averaged for three mice from each group.*

The analogous data for *O*- and *S*-linked GM_2 analogues is shown in Figure 5.12. Antigen specific IgG titres are high and similar. In addition, recognition of *S*-glycoside

by *O*-glycoside derived IgG is equivalent or higher than binding to the immunizing antigen. As mentioned previously, cross reactivity of sera against *S*-glycosides with *O*-GM_2 was not appreciable.

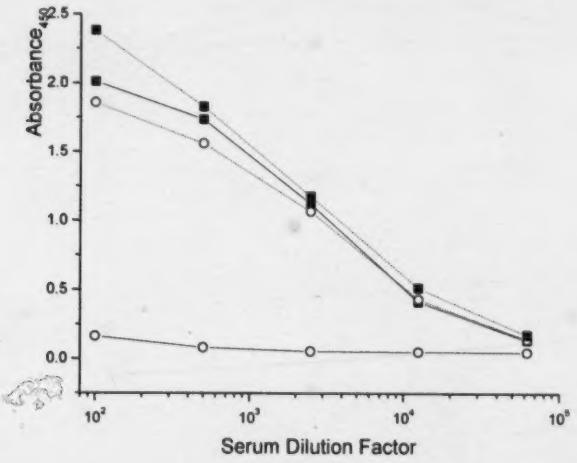


Figure 5.12. *Binding to immobilized S-GM_2-BSA* ***132*** *(■) and O-GM_2-BSA* ***133*** *(○) by murine IgG generated from immunization with S-GM_2-TT* ***129*** *(—) or O-GM_2-TT* ***130*** *(---). Data are averaged for three mice from each group.*

In an effort to further investigate the conformational implications of the thioglycosidic linkage, a commercial monoclonal antibody (GMR-6 IgM;[271] Seikagu, Japan) against GM_3 was probed for its ability to bind immobilized *O*- and *S*-linked trisaccharide-BSA conjugates **127** and **128** by indirect ELISA. Although only weak titres were observed, it was evident that the *O*-glycoside enjoyed greater recognition (Figure 5.13).

It was also of interest to determine whether or not the antibodies produced against the thiooligosaccharides would recognize gangliosides in a cellular setting. The B16-F10 melanoma cell line is known to express high levels of ganglioside GM_3. A B16-F10

melanoma cell line was obtained from Dr. Mavanur Suresh at the University of Alberta. A suspension of the cells were transferred to microtitre wells at various dilutions and

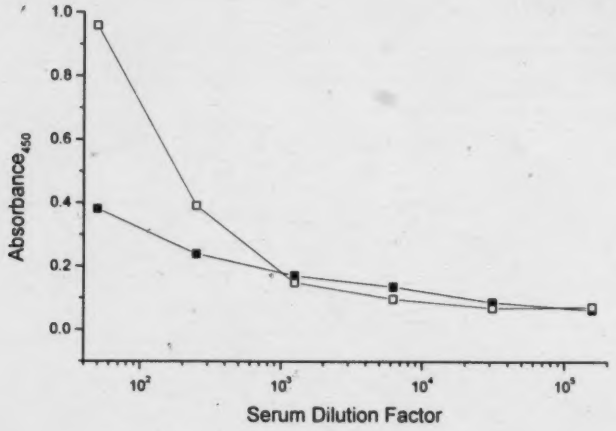


Figure 5.13. *Titration of monoclonal IgM antibody GMR-6 against S-GM3-BSA (**127**; ■) and O-GM3-BSA (**128**; □).*

ELISA was performed as described previously for the BSA-ganglioside conjugates. However, this process disrupted adhesion of the cells to the plate and no meaningful data were obtained. Chemical fixation of cells to the plate might provide an opportunity to evaluate antibody binding in the future.

5.6 *S*-Glycosides as immunogens: Speculation and conclusions

Several conclusions can be drawn with regard to these data. First, it is apparent that the synthetic ganglioside-protein conjugates each generate a carbohydrate specific immune response in the majority of the mice immunized. Although more of the animals responded to *S*-glycosides in each case, the sample number is too small to make serious inferences regarding their relative capacities to induce an IgG response. Analysis of the immune sera that did contain antigen specific IgG reveal that the response to immunization with thiooligosaccharide-protein conjugates is either superior or at least

equivalent to that observed following immunization with the native oligosaccharide. It is also clear that either class of glycoside generates a paratope capable of accommodating the other glycoside. An exception to the observed trend of cross reactivity is noted following immunization with the *S*-linked tetrasaccharide, as antibodies were not observed to bind to the *O*-glycoside. In the three out of four instances where recognition of the heterogeneous glycoside was evident, the IgG titres were comparable to the antigen specific response to the *O*-glycoside.

The failure of antibodies to *S*-GM_2 to cross react with *O*-GM_2 may be rationalized in terms of the flexibility of the thioglycosidic linkage. Conformational studies of the naturally occurring glycolipid have identified a defined arrangement of the terminal GalNAc and Neu5Ac residues, stabilized by inter-residue contacts. Solution NMR and molecular modelling studies have identified a single low energy conformation for the Neu5Acα(2→3)[GalNAcβ(1→4)]Gal trisaccharide portion of glycolipid GM_2.[108-110] Variation of the spacing between the Neu5Ac and Gal residues by substitution of the glycosidic oxygen atom with sulfur could lead to changes in these stabilizing forces. Any disruptions of the inter-residue are more likely to impact on the conformation of the Neu5Ac-Gal linkage owing its enhanced flexibility relative to the GalNAc-Gal linkage, which has been identified as being particularly rigid.[110] The antigen combining sites likely reflect the dominant conformation of the *S*-glycoside, which may not be readily adopted by the *O*-glycoside. Likewise, the higher capacity of *O*-glycoside derived antibodies to accommodate *S*-glycosides is not unexpected given the relative readiness with which *S*-glycosides might undergo conformational change about the Neu5Acα(2→3)Gal thio-linkage. While the predominant antigenic conformation of the

Neu5Ac-Gal disaccharide in *S*-GM_2 appears to differ from its counterpart, its recognition by *O*-glycoside derived antibodies suggests common bound conformations are accessible.

By definition, polyclonal sera contain antibodies against a variety of epitopes. The importance of the terminal sialic acid containing region in some of the epitopes is demonstrated by the high titres observed against the Neu5Acα(2→3)Gal-BSA conjugate **136**, and by the failure of sera against *S*-GM_2 glycoconjugate **129** to recognize it *O*-linked counterpart **133**. Clearly some of the antibodies to each of these immunogens recognize the reducing terminal portion of the tri- and tetra-saccharides, which is evident in the recognition of modified glucosyl ceramide and lactosyl ceramide. The recognition of the glucose containing conjugate is likely overestimated as a consequence its relatively high epitope density (n = 20).

The failure of mono- and disaccharides examined to inhibit binding of sera to the *O*-GM_3-BSA conjugate is troubling. Oligosaccharide antigenic determinants normally correspond to di- to tetra-saccharide sequences.[272] It is certainly conceivable that the mono- and disaccharides examined are low affinity ligands because of their size. Cooperative binding by the two antigen combining sites of an IgG molecule might account for recognition of the same molecules in the context of immobilized BSA conjugates.

The results obtained in these studies are in accord with those found in our investigation of thiooligosaccharide analogues of two microbial carbohydrate antigens, a β1,2-mannan component of *Candida albicans* cell wall and a trisaccharide from the variant Y *Shigella flexneri* O-polysaccharide (Figure 5.14).[259] BSA or TT conjugates of

these oligosaccharides were evaluated in mice or rabbits and were found to generate an antigen specific immune response that in one case approached, and in two others exceeded the response to the native oligosaccharide. Antibodies against the *S*-glycosides demonstrated a clear cross reactivity with the corresponding *O*-glycosides, and vice-versa. These findings are indicative of a certain degree of conformational similarity between the related structures.

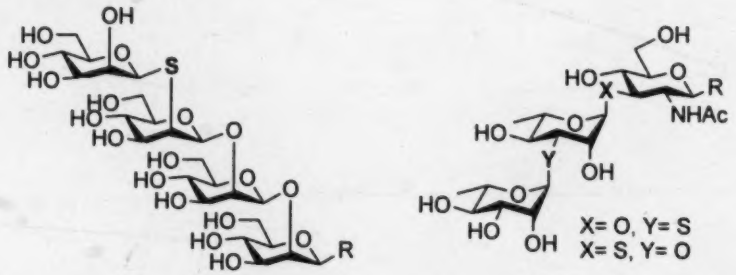


Figure 5.14. *Thiooligosaccharide analogues of microbial antigens used as immunogens.*

While it is not at all clear that *S*-glycosides would be generally superior immunogens to *O*-glycosides after taking into account the diminished avidity of the sera towards the native structures, some antigens that suffer from *in vivo* enzymatic degradation may enjoy a relative boost in immunogenicity after modification of the glycosidic linkage. The finding that immunoglobulin derived from *O*-antigens binds reasonable well to thiooligosaccharide structures suggest that these molecules may also find application as therapeutics. For example, pathological anti-ganglioside antibodies present in the sera of patients with auto-immune diseases such as Guillian-Barré syndrome may bind to *S*-linked gangliosides, which could thereby serve as stable immunoabsorbents.[273]

Chapter Six
Conclusions and Future Directions

The research described in this thesis describes significant synthetic challenges in oligosaccharide synthesis and subsequently novel biological application of the thiooligosaccharides that were the primary focus of the synthetic work. Significant contributions to the field of thiosialoside synthesis have come from the groups of Hasegawa, Schmidt, and von Itzstein in the past. Despite their considerable achievements, the establishment of the Neu5Acα(2→3[*S*])GalβOR linkage remained a considerable challenge, which had not been investigated systematically prior to this study.[274]* The importance of protecting groups to the success of *gulo*- to *galacto*-epimerization reactions has been recognized, as have the nature of some of the attendant side reactions involved. A method for the synthesis of the previously inaccessible, versatile synthon 3'-thiolactose octaacetate was established.[275]

Chemical and chemoenzymatic elaboration of thiosialyl lactosides and galactosides permitted access to higher thiooligosaccharides for incorporation into ganglioside glycoconjugates. Two glycosyltransferases were found to utilize thiosialyl oligosaccharide substrates. Taken together, these transformations,[276] the galactosylation of Galα(1→3[*S*])Galβ(1→4)GlcβOR by α(1,3)GalT,[275] two earlier studies,[206,207] and the absence of contradictory reports suggest that this may be a general phenomenon. To evaluate the utility of *S*-glycosides as immunogens, the analogous *O*-glycosides were also synthesized chemoenzymatically. Glycosyltransferase mediated synthesis was

* Concurrent with our investigation, von Itzstein and co-workers published an insightful study in this regard. See reference 157.

facilitated by enzymatic preparation of sugar nucleotides by use of both epimerase and synthetase functions.

Our report details the first examples of glycosyltransferase catalyzed construction of thiooligosaccharides.[275] However, in contrast to glycosylation of oligosaccharides containing existing thioglycosidic linkages, further efforts to utilize carbohydrate thiols as acceptors were unsuccessful. The synthetic thiol containing substrates evaluated may find further use as tools in exploration of the acceptor specificities of a number of different enzymes. When successful, the synthesis of a thiol containing oligosaccharide also provides a "handle" for modification of the structure based on the differing reactivity of the thiol and hydroxyl functions. If structural and mechanistic understanding of the glycosyltransferases can approach the level of understanding now held for the glycoside hydrolases, one can envision a protein engineering approach to "thioglycosyltransferases".

Synthetic oligosaccharide analogues were constructed on a truncated sphingosine core. The thioglycosides, with putative enhancements in metabolic stability, were conjugated to TT and along with two thioglycoside analogues of microbial structures were for the first time evaluated as antigens *in vivo*.[259] Direct comparison of the results to those obtained following immunization of mice with *O*-glycosides allowed several conclusions. Conjugate vaccines containing *S*-linked immunogens generated an antigen specific immune response, which was comparable to the immune response to *O*-glycosides. Furthermore, the antibodies produced to *S*-glycosides were cross reactive with the analagous *O*-glycosides and related structures, suggesting a degree of conformational similarity. Among the four *S*-linked immunogens evaluated to date, only

the branched thiooligosaccharide (*S*-GM_2) failed to elicit IgG antibody paratopes complementary to its *O*-linked counterpart. This result is likely explained by the relative flexibilities of the glycosidic linkages of the adjacent *O*- and *S*-linked residues. The contention would be supported by recognition of *S*-GM_2 by a monoclonal antibody to asialo-GM_2.* In light of the work described herein and evaluation of other thiooligosaccharide immunogens, it can be anticipated that antigens bearing critical residues attached via hydrolysis resistant linkages could find application either in active immunotherapy or as ligands for immunoabsorption of neuropathy associated antibodies directed against self oligosaccharides.

While the immunological results imply certain differences and similarities between the bound and antigenic conformations of *S*- and *O*-linked sialosides, these are largely assumptions. No studies have appeared which compare the conformations of *O*- and *S*-sialosides containing a single thioglycosidic linkage. The conformational analysis of such molecules is complicated by the increased inter-residue distance across the thioglycosidic linkage, but is feasible.[277] A number of techniques are also available for the comparison of the bound conformations of such molecules.

A logical extension of this work would involve the synthesis and evaluation of glycoconjugates containing α(2→8*S*)-linked terminal disialosyl residues and an analogue of GM_2 containing an *S*-linked GalNAc residue. Both of these directions would benefit from work reported in this thesis and would provide molecules with a variety of uses, ranging from non-hydrolysable substrates for crystallographic studies to immunogens to ligands for affinity chromatography or affinity based removal of autoantibodies in

* The murine mAb 2D4 against asialo GM_2 is available from the American Type Culture Collection (ATCC TIB 185)

established diseases. Although *S*-linked sialosides have been shown to resist degradation by enzymes of bacterial origin, the stability of these molecules and *O*-gangliosides in human serum remains an open question.

Chapter Seven

Experimental Section

General:

Chemical Synthesis: All chemical reagents were of analytical grade, used as supplied from Sigma-Aldrich unless indicated. Solvents used in non-hydrolytic reactions were distilled under an inert atmosphere, except *N,N*-dimethylformamide (stored over 3Å molecular sieves and subjected to reduced pressure for ~fifteen minutes (<0.5mm Hg) prior to use) and pyridine (dried over solid potassium hydroxide and used without further purification). Unless otherwise noted, all reactions were carried out at room temperature and non-hydrolytic reactions were performed under a positive pressure of argon. Solvents were removed at between 20 and 40 °C (bath temperature) unless indicated. Molecular sieves were stored in an oven (>170 °C) and cooled *in vacuo* prior to use. Ammonia was distilled once from sodium metal prior to use. Rexyn-101 (H^+ form) was used where acidic ion-exchange resin is indicated. Analytical thin layer chromatography (TLC) was conducted on silica gel 60-F_{254} (Merck). Plates were visualized under UV light, and/or by treatment with either acidic cerium ammonium molybdate or 5% ethanolic sulfuric acid, followed by heating.

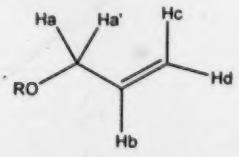
Enzymatic Synthesis: Clones of the following recombinant enzymes CMP-Neu5Ac synthetase from *N.meningitidis*,[215] UDP *N*-acetylglucosamine 4-epimerase from *C.jejuni* NCTC 11168,[212] β(1,4) *N*-acetylgalactosaminyl transferase from *C.jejuni* O:36,[213] α(2,3) sialyltransferase[217] and α(2,3/8) sialyltransferase[213] from *Campylobacter jejuni* OH4384 were expressed in *E. coli*, isolated by separation of the

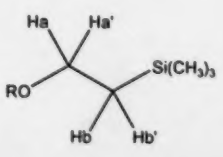
pellet and supernatant following cell lysis, diluted or resuspended in the appropriate buffer and used without further purification. The activity of the α(2,3) sialyltransferase and β(1,4) *N*-acetylgalactosaminyl transferases were determined by radiochemical assay using 8-methoxylcarbonyloctyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside and 8-methoxylcarbonyloctyl (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside, respectively, as acceptor substrates.[241] CMP-Neu5Ac was prepared as described.[215] Following removal of the protein by centrifugation of the CMP-Neu5Ac containing solution, the supernatant was lyophilized and the crude solid obtained was used directly in reactions assuming 90% conversion of starting material. Recombinant α(1,3)GalT[226] was purified by loading *E. coli* extracts onto an SP-Sepharose Fast Flow column equilibrated with 50 mM MOPS, pH 7.0, containing 1 mM dithiothreitol, washing the column with equilibration buffer to background levels of protein and then eluting the enzyme with equilibration buffer containing 0.4 M NaCl. Recombinant β(1,3)GlcNAcT[249] was isolated on a UDP-hexanolamine column. Enzyme activity was deterimined by radiochemical assay using β-octyl lactose as an acceptor.[241] Assays were conducted using final concentrations of 0.5 mM donor and either 2 mM (α1,3-GalT) or 10mM (β1,3-GlcNAcT) acceptor. A unit of enzyme activity is the amount that catalyzes the formation of 1 μmol of product per minute at 37 °C using the specified acceptor. Alkaline phosphatase was obtained from Roche Diagnostics.

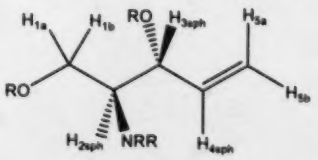
Chromatography: Medium pressure chromatography was conducted using silica gel (230-400 mesh, SiliCycle, Montreal) or Iatrobeads 6RS-8060 (Iatron Laboratories Inc., Japan), using a ratio between 50:1 and 125:1 (w/w) of silica gel:crude product at flow

rates of 5-10 mL min^{-1}. Linear gradients were generated using a Q-Grad gradient mixer from Lab Alliance (State College, PA, USA). Reverse phase C_{18} silica gel cartridges (tC_{18} SepPak) were obtained from Waters Corp. (Milford, USA). HPLC purification was conducted on a Waters Delta 600 system using a UV absorbance detector. Separations were performed on Beckman C_{18}-silica semi-preparative, Kromasil C_{18} semi-preparative or Hamilton PRP-1 reverse phase semi-preparative columns as noted, with combinations of water and methanol (flow rate 1.5-2.5 mL min^{-1}) as eluents.

Analytical: ^{1}H and ^{13}C NMR spectra were recorded on Varian INOVA 400, 500 and 600 MHz spectrometers. ^{1}H NMR chemical shifts are reported in δ (ppm) units using signals from residual solvents as a reference. ^{13}C NMR chemical shifts are referenced to internal solvent or to external acetone in the case of D_2O. ^{1}H and ^{13}C NMR spectra were tentatively assigned with the assistance of COSY, HMQC, HMBC, TOCSY and TROESY spectra as necessary. Where $^{2}J_{H,H}$ and $^{3}J_{H,H}$ values are not indicated for carbohydrate resonances of defined multiplicity, the values were found to be in accord (± 0.2 Hz) with the value reported for the same coupling constant observed for a related resonance. NMR data for compounds containing sphingosine or ceramide and derivatives as the aglycon were labelled as indicated below. A labelling scheme for hydrogen atoms of the allyl and 2-trimethylsilyl(ethyl) anomeric protecting groups were is also provided.







Electrospray ionization mass spectra were recorded on a Micromass Zabspec TOF mass spectrometer. For high resolution mass determination, spectra were obtained

by voltage scan over a narrow range at a resolution of approximately 10^4. MALDI TOF mass spectra were recorded on a Voyager Elite spectrometer from Applied Biosystems. All spectra were acquired at the mass spectrometry laboratory in this department. Optical rotations were determined with a Perkin-Elmer model 241 polarimeter at 22±2 °C, and are reported in units of degr. mL g^{-1} dm^{-1}. Elemental analysis and optical rotations were performed by the analytical services facility at this department.

Immunization: 8 week old Balb/c mice were immunized by interperitoneal injection of tetanus toxoid conjugates (50μg) in a 1:1 mixture of PBS and Complete Freund's Adjuvant. Subsequent injections (50μg) on days 21 and 28 were made subcutaneously in PBS and Incomplete Freund's Adjuvant. Mice were bled on day 38 and sera were isolated by centrifugation and then frozen at –20 °C.

Enzyme Linked Immunosorbent Assay: Titrations. Carbohydrate-protein conjugates (10 μg/mL; 100 μL; 4 °C, overnight) were used to coat 96-well ELISA plates (MaxiSorp, Nunc). The plate was washed (Molecular Devices Skan Washer 400) 5 times with PBST (PBS containing Tween 20, 0.05% v/v). Mouse sera were diluted with PBST containing 0.15 BSA and the solutions were added to the plate and incubated at room temperature for 2 hours. The plate was washed with PBST (5 times) and goat anti-mouse IgG antibody conjugated to horseradish peroxidase (Kirkegaard & Perry Laboratories; 1:2000 dilution in PBST) was added (100 μL) and incubated for a period of 1 hour. The plate was washed 5 times with PBST before addition of a 1:1 mixture of 3,3′,5,5′-tetramethylbenzidine (0.4 g/L) and 0.02% H_2O_2 solution (Kirkegaard & Perry Laboratories; 100 μL). After 2 minutes the reaction was stopped by addition of 1M phosphoric acid (100 μL). Absorbance was read at 450 nm (Molecular Devices Spectra

Max 190 plate reader). Monoclonal antibody GMR-6 was purchased from Seikagaku Corp. (Tokyo, Japan). **Inhibition experiments.** The sera were mixed with inhibitor dissolved in PBST at varying concentrations and the resulting solutions were added to the coated microtitre plate (see above) in triplicate and incubated at room temperature for 18 h. The plate was washed with PBST (5 times), and goat anti-mouse (IgG) antibody conjugated to horseradish peroxidase (diluted 1:2000, Kirkegaard & Perry Laboratories) in PBST (100 µl) was added and incubated for 1 h at room temperature. The plate was washed with PBST (5 times); 3,3′,5,5′-tetramethylbenzidine (100 µl, Kirkegaard &Perry Laboratories) was added, and after 2 min the color reaction was stopped by the addition of 1 M phosphoric acid (100 µl).

Compounds:

(2,3-Di-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-1,2,3,6-tri-*O*-acetyl-α/β-D-glucopyranose (3)

A solution of lactose (10.270 g; 28.5 mmol), benzaldehyde dimethyl acetal (15 mL; 99.9 mmol; 3.5 eq.) and *p*-toluenesulfonic acid monohydrate (0.180 g) in *N,N*-dimethylformamide (100 mL) was stirred for 18 hours at 60 °C. After cooling, water (150 mL) was added and the mixture was extracted with hexanes (3 x 100 mL). The aqueous layer was concentrated and then evaporated from ethanol (100 mL), followed by toluene (100 mL). The solid material obtained was acetylated by addition in portions to a refluxing mixture of sodium acetate (11.4 g; 139 mmol; 4.9 eq.) in acetic anhydride (100 mL; 1.060 mol; 37.2 eq.). Following concentration of the solution, the residue was chromatographed on silica gel with a mixture of toluene/acetone/hexanes (6:2:1) as eluent to afford **3** as a mixture of anomers (9.2 g; 13.5 mmol; 47%). A small portion of this material was crystallized from ethyl acetate to afford a colourless powder (5:1 α/β):

^{1}H NMR for the α anomer (500 MHz, $CDCl_3$) δ 7.49-7.36 (m, 5H, Ar), 6.26 (d, 1H, H-1, $J_{1,2}$ = 3.7 Hz), 5.49 (dd, 1H, H-3, $J_{3,2} \sim J_{3,4} \sim$ 9.7 Hz), 5.48 (s, 1H, C*H*Ph), 5.28 (dd, 1H, H-2', $J_{2',1'}$ = 8.0 Hz, $J_{2',3'}$ = 10.2 Hz), 5.03 (dd, 1H, H-2), 4.89 (dd, 1H, H-3', $J_{3',4'}$ = 3.6 Hz), 4.50-4.45 (m, 2H, H-1', H-6a), 4.34 (bd, 1H, H-4'), 4.30 (dd, 1H, H-6a', $J_{6a',5'}$ = 1.3 Hz, $J_{6a',6b'}$ = 12.6 Hz), 4.14 (dd, 1H, H-6b, $J_{6b,5}$ = 4.5 Hz, $J_{6b,6a}$ = 12.3 Hz), 4.08-3.99 (m, 2H, H-6b', H-5), 3.82 (dd, 1H, H-4, $J_{4,5} \sim$ 9.8 Hz), 3.47 (bs, 1H, H-5'), 2.18, 2.12 (2s, 6H, 2xC(O)CH_3), 2.05 (s, 6H, 2xC(O)CH_3), 2.04, 2.00 (2s, 6H, 2xC(O)CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7, 170.2, 170.0, 169.9, 169.0, 168.9, 137.4, 129.2, 128.2, 126.5, 101.4, 101.1, 89.0, 75.5, 73.1, 72.1, 71.0, 69.4, 69.2, 69.0, 68.4, 66.5, 61.5, 20.95, 20.83, 20.80, 20.70, 20.69, 20.5; ESI HRMS calc'd for $C_{31}H_{38}O_{17}Na$: 705.2001. Found: 700.1999; Anal. calc'd for $C_{31}H_{38}O_{17}$: C 54.54, H 5.61. Found: C 54.50, H 5.61.

(2,3-Di-*O*-acetyl-4-*O*-benzoyl-6-bromo-6-deoxy-β-D-galactopyranosyl)-(1→4)-1,2,3,6-tri-*O*-acetyl-α/β-D-glucopyranose (4)

The lactose hexaacetate **3** (2.989 g; 4.38 mmol; 3:1 β/α) was combined with barium carbonate (2.680 g; 13.57 mmol; 3.1 eq.) , carbon tetrachloride (100 mL), and *N*-bromosuccinimide (1.091 g; 6.13 mmol; 1.4 eq.) in a dry flask fitted with a reflux condenser. The flask was immersed in an oil bath pre-equilibrated at 90 °C. After 2 hours, the reaction mixture was cooled to room temperature, filtered through Celite, and evaporated to dryness. Chromatography of the residue on silica gel with ethyl acetate/hexanes (2:1) as eluent afforded **4** as an anomeric mixture (2.767 g; 3.64 mmol; 83 %): ^{1}H NMR for the β anomer (500 MHz, $CDCl_3$) δ 8.10-8.02 (m, 2H, Ar), 7.66-7.59 (m, 1H, Ar), 7.55-7.46 (m, 2H, Ar), 5.77 (d, 1H, H-4', $J_{4',3'}$ = 3.2 Hz), 5.70 (d, 1H, H-1, $J_{1,2}$ = 8.3 Hz), 5.31 (dd, 1H, H-2', $J_{2',1'}$ = 7.8 Hz, $J_{2',3'}$ = 10.3 Hz), 5.11-5.05 (m, 2H, H-2,

H-3'), 4.58 (d, 1H, H-1'), 4.49 (dd, 1H, H-6a, $J_{6a,5}$ = 1.8 Hz, $J_{6a,6b}$ = 12.3 Hz), 4.15 (dd, 1H, H-6b, $J_{6b,5}$ = 4.8 Hz), 3.95-3.78 (m, 3H, H-4, H-5', H-5), 3.40-3.34 (m, 2H, H-6a', H-6b'), 2.13, 2.10 (2s, 6H, 2xC(O)CH_3), 2.04 (s, 9H, 3xC(O)CH_3), 1.93 (s, 3H, C(O)CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3, 170.1, 169.55, 169.50, 168.92, 168.86, 165.4, 133.8, 130.0, 128.76, 128.71, 100.58, 91.64, 75.3, 74.0, 73.6, 72.6, 71.2, 70.5, 69.2, 67.8, 61.7, 27.7, 20.86, 20.85, 20.80, 20.6 (2C), 20.5; ESI HRMS calc'd for $C_{31}H_{37}BrO_{17}Na$: 783.1112. Found: 783.1120; Anal. calc'd for $C_{31}H_{37}BrO_{17}$: C 48.89, H 4.90. Found: C 49.38, H 5.01.

(2,3-Di-*O*-acetyl-6-*S*-acetyl-4-*O*-benzoyl-6-thio-β-D-galactopyranosyl)-(1→4)-1,2,3,6-tri-*O*-acetyl-α/β-D-glucopyranose (5)

The bromide **4** (0.165 g; 0.217 mmol) was combined with potassium thioacetate (0.050 g; 0.433 mmol; 2.0 eq.) and *N*,*N*-dimethylformamide (4.0 mL) was added. The mixture was heated at 50 °C for 20 hours, diluted with ethyl acetate (20 mL), washed with water (2 x 10 mL), dried over sodium sulfate, filtered and concentrated. Chromatography of the residue on a column of silica gel capped by a 1 cm plug of charcoal afforded **5** (0.150 g; 0.197 mmol; 91%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10-8.05 (m, 2H, Ar), 7.66-7.60 (m, 1H, Ar), 7.54-7.48 (m, 2H, Ar), 5.71 (d, 1H, H-1, $J_{1,2}$ = 8.3 Hz), 5.65 (d, 1H, H-4', $J_{4',3'}$ = 3.3 Hz), 5.31 (dd, 1H, H-3, $J_{3,2} \sim J_{3,4} \sim$ 9.2 Hz), 5.18 (dd, 1H, H-2', $J_{2',1'}$ = 7.9 Hz, $J_{2',3'}$ = 10.3 Hz), 5.10 (dd, 1H, H-2, $J_{2,3}$ = 9.4 Hz), 5.03 (dd, 1H, H-3'), 4.53 (d, 1H, H-1'), 4.48 (dd, 1H, H-6a, $J_{6a,5}$ = 2.0 Hz, $J_{6a,6b}$ = 12.3 Hz), 4.14 (dd, 1H, H-6b, $J_{6b,5}$ = 5.0 Hz), 3.91 (dd, H-4, $J_{4,5}$ = 9.8 Hz), 3.82-2.73 (m, 2H, H-5, H-5'), 3.11 (dd, 1H, H-6a', $J_{6a',5'}$ = 6.9 Hz, $J_{6a',6b'}$ = 13.9 Hz), 3.00 (dd, 1H, H-6b', $J_{6b',5'}$ = 7.4 Hz), 2.36, 2.13, 2.11, 2.05, 2.04, 2.03, 1.92 (7s, 21H, 7xC(O)CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 194.5, 170.3,

170.1, 169.6, 169.5, 168.94, 168.86, 165.5, 133.7, 129.9, 128.9, 128.7, 100.6, 91.7, 75.2, 73.6, 72.56, 72.53, 71.3, 70.6, 69.4, 68.2, 61.7, 30.5, 28.7, 21.0, 20.8 (2C), 20.6 (2C), 20.5; ESI HRMS calc'd for $C_{33}H_{40}O_{18}SNa$: 779.1828. Found: 779.1826; Anal. calc'd for $C_{33}H_{40}O_{18}S$: C 52.38, H 5.33. Found: C 52.02, H 5.53.

2-(Trimethylsilyl)ethyl (2,3-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (6)

2-(Trimethylsilyl)ethyl (2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside[122] (3.260 g; 4.4 mmol) was combined with 80% acetic acid (80 mL) and heated at 50 °C for nine hours, then concentrated. Recrystallization of the residue from hexanes/ethyl acetate gave the diol **6** (2.470 g; 3.8 mmol; 86%). All spectral data matched those reported earlier for synthesis of this compound under different conditions.[122]

2-(Trimethylsilyl)ethyl (2,3-di-*O*-acetyl-6-*O*-trifluoromethanesulfonyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (7)

The diol **6** (0.310 g; 0.47 mmol) was dissolved in dichloromethane (8.0 mL) and pyridine (0.150 mL; 1.85 mmol; 3.9 eq.) with stirring under argon, and was then cooled to –30 °C. Trifluoromethanesulfonic anhydride (0.088 mL; 0.52 mmol; 1.1 eq.) was added dropwise over a one minute period, and the mixture was stirred for one hour, then diluted with dichloromethane (25 mL), washed successively with 1M hydrochloric acid, saturated sodium bicarbonate solution, and water, then dried over sodium sulfate, filtered and concentrated. The residue was chromatographed on silica gel using hexanes/ethyl acetate (3:2) as eluent affording the title compound **7** (0.264 g; 0.34 mmol; 71%): $[\alpha]_D$ –16 (c 0.55, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 5.19 (dd, 1H, H-3, $J_{3,2} = J_{3,4} = 9.3$ Hz), 5.14

(dd, 1H, H-2', $J_{2',1'}$ = 7.8 Hz, $J_{2',3'}$ = 10.1 Hz), 4.93 (dd, 1H, H-3', $J_{3',4'}$ = 3.3 Hz), 4.86 (dd, 1H, H-2, $J_{2,1}$ = 7.9 Hz), 4.67 (dd, 1H, H-6a', $J_{6a',6b'}$ = 10.6 Hz, $J_{6a',5'}$ = 5.5 Hz), 4.61 (dd, 1H, H-6b', $J_{6b',5'}$ = 6.9 Hz), 4.51 (d, 1H, H-1'), 4.48 (dd, H-6a, $J_{6a,5}$ = 12.0 Hz, $J_{6a,6b}$ = 2.1 Hz), 4.45 (d, 1H, H-1), 4.10 (dd, 1H, H-6b, $J_{6b,5}$ = 5.1 Hz), 4.06 (d, 1H, H-4', $J_{4',3'}$ = 2.7 Hz), 3.91 (m, 1H, H-a), 3.85 (m, 1H, H-5'), 3.81 (dd, 1H, H-4, $J_{4,3} = J_{4,5}$ = 9.3 Hz), 3.60 (m, 1H, H-5), 3.53 (m, 1H, H-a'), 2.58 (bs, 1H, OH-4'), 2.09, 2.06, 2.03, 2.01, 2.00 (5 x s, 15H, C(O)C*H*$_3$), 0.90 (m, 2H, H-b, H-b'), -0.05 (s, 9H, Si(CH3)3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 170.0, 169.8, 169.4, 169.2, 118.6 (q, CF_3, $^1J_{C,F}$ = 320 Hz), 100.1 , 99.9 , 75.7, 72.8, 72.7, 72.6, 72.5, 71.9, 71.8, 69.2, 67.4, 66.3, 62.1, 20.75, 20.68, 20.56 (3 x C), 17.8, -1.5; ESI HRMS calc'd for $C_{28}H_{43}F_3O_{18}SSiNa$: 807.1784. Found: 807.1783. ^{1}H NMR and mass spectra were recorded for a side product, **2-(trimethylsilyl)ethyl (2,3-di-*O*-acetyl-4,6-di-*O*-trifluoromethanesulfonyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.18 (bd, 1H, H-4', $J_{4',3'}$ = 1.2 Hz), 5.17 (dd, 1H, H-3, $J_{3,2} = J_{3,4}$ = 9.2 Hz), 5.10-5.08 (m, 2H, H-2', H-3'), 4.84 (dd, 1H, H-2, $J_{2,1}$ = 8.0 Hz), 4.66-63 (m, 1H, H-1'), 4.59 (dd, 1H, H-6a', $J_{6a',6b'}$ = 10.9 Hz, $J_{6a',5'}$ = 7.1 Hz), 4.49-4.43 (m, 3H, H-1, H-6a, H-6b'), 4.10-4.04 (m, 2H, H-6b, H-5), 3.94-3.88 (m, 1H, H-a), 3.84 (dd, 1H, H-4, $J_{4,5}$ = 9.6 Hz), 3.59 (ddd, 1H, H-5, J = 2.1 Hz, J = 4.9 Hz), 3.56-3.50 (m, 1H, H-a'), 2.08, 2.07, 2.03, 2.00, 1.98 (5s, 15H, 5xC(O)CH_3), 0.93 (ddd, 1H, H-b, J = 6.6 Hz, J = 10.5 Hz, J = 14.0 Hz), 0.85 (ddd, 1H, H-b', J = 5.8 Hz, J = 10.1 Hz, J = 14.0 Hz), -0.03 (s, 9H, Si(CH3)3); ESI HRMS calc'd for $C_{29}H_{42}O_{20}F_6NaS_2Si$: 939.1277. Found: 939.1277.

2-(Trimethylsilyl)ethyl *S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonate)-(2→6)-(2,3-di-*O*-acetyl-6-thio-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (8)

2-(Trimethylsilyl)ethyl (2,3-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside **6** (0.240 g; 0.37 mmol) was dissolved in freshly distilled dichloromethane (5.0 mL) and pyridine (0.119 mL; 1.47 mmol; 4 eq.) with stirring under argon. After cooling the solution to –25°C, trifluoromethanesulfonic anhydride (0.074 mL; 0.44 mmol; 1.2 eq.) was added dropwise over approximately one minute. After stirring at constant temperature for 45 minutes, the reaction mixture was diluted with dichloromethane (30 mL), washed successively with 1M HCl, saturated $NaHCO_3$ solution, and water. The organic layer was dried over sodium sulfate, filtered, and concentrated. The crude residue was combined with methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-*S*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonate[116] (0.202 g; 0.37 mmol; 1.0 eq.), and the mixture was dissolved in *N,N*-dimethylformamide (3.0 mL) under argon. After cooling to -25°C, diethylamine (0.380 mL, 3.7 mmol, 10eq.) was added and the solution was stirred for 2 hours, after which time it was concentrated under reduced pressure. Column chromatography of the residue in methanol/dichloromethane (3:97) afforded **8** (0.301 g; 0.27 mmol; 73 %) and a small amount of **9**[115] (0.031 g; 0.26 mmol; 7%). Data for **8**: $[\alpha]_D$ 2 (c 0.94, CHCl3); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.29 (dd, 1H, H-7", $J_{7",8"}$ = 9.6 Hz $J_{7",6"}$ = 2.0 Hz), 5.21 (m, 1H, H-8"), 5.15-5.08 (m, 3H, N*H*Ac, H-2', H-3), 4.93-4.87 (m, 3H, H-4", H-3', H-2), 4.58 (d, 1H, H-1', $J_{1',2'}$ = 7.91 Hz), 4.46-4.43 (m, 2H, H-1, H-6a), 4.26 (dd, 1H, H-9a", $J_{9a",8"}$ = 2.88 Hz, $J_{9a",9b"}$ = 12.5 Hz) 4.19 (d, 1H, H-4', $J_{4',3'}$ = 3.2 Hz), 4.15-4.08 (m, 2H, H-9b", H-6b), 3.95-3.88 (m, 2H, H-5", H-a), 3.83-3.77 (m, 5H, CO_2CH_3, H-6", H-4), 3.62-3.58

(m, 2H, H-5', H-5), 3.53 (ddd, 1H, H-a'), 2.85 (dd, 1H, $J_{6a',6b'}$ = 14.3 Hz, $J_{6a',5'}$ = 7.2 Hz, H-6a'), 2.78 (dd, H-6b', $J_{6b',6a'}$ = 14.3 Hz, $J_{6b',5'}$ = 6.9 Hz), 2.73 (dd, H-3"eq, $J_{3"eq,3"ax}$ = 12.8 Hz, $J_{3"eq,4"}$ = 4.6 Hz), 2.18-2.00 (27H, 9 x OC(O)CH_3), 1.96 (dd, 1H, H-3_{ax}, $J_{3"ax,4"}$ = 12.5 Hz), 1.87 (s, 3H, NHC(O)CH_3), 0.89 (m, 2H, H-b, H-b'), -0.03 (s, 9H, Si(CH_3)$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7, 170.6, 170.28, 170.23, 170.0 (2 x C), 169.9, 169.7, 169.5, 169.2, 168.1, 100.2 , 100.0 , 83.8, 73.95, 73.87, 73.4, 73.0, 72.7, 71.8, 69.6, 69.3, 68.1, 67.3, 66.7, 62.6, 62.0, 53.3, 49.6, 38.1, 29.8, 23.3, 21.3, 21.1, 20.90, 20.87 (multiple C), 20.81, 20.76, 17.98, -1.3; ESI MS calc'd for $C_{47}H_{71}NO_{27}SSiNa$: 1164. Found: 1164. Anal. calc'd for $C_{47}H_{71}NO_{27}SSi$: C 49.42, H 6.27, N 1.24. Found: C 49.60, H 6.25, N 1.40. Alternatively, direct acetylation of the crude reaction mixture afforded **9** in 83% yield after chromatography. All spectral data were found to be in accord with those reported previously. Removal of the 2-(trimethylsilyl)ethyl protecting group and generation of the hemiacetal proceed smoothly under previously reported conditions.[115]

Methyl 2,4,6-tri-*O*-benzyl-β-D-gulopyranoside (10)

Methyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside[132,133] (0.229 g; 0.49 mmol) was dissolved in dichloromethane (3.0 mL) and pyridine (0.160 mL; 1.97 mmol; 4.0 eq.) with stirring under argon at –35 °C. Trifluoromethanesulfonic anhydride (0.165 mL; 0.98 mmol; 2.0 eq.) was added to the solution dropwise over a period of approximately 30 seconds. After 10 minutes, the reaction mixture was diluted with dichloromethane (15 mL), washed with water (10 mL), 1M HCl (10 mL), followed by water (10 mL), dried over sodium sulfate, filtered and concentrated. The residue was dissolved in *N,N*-dimethylformamide (3.0 mL) and tetrabutylammonium nitrite (0.300 g; 1.1 mmol; 2.2

eq.). After stirring overnight at room temperature, no starting material remained. The solution was concentrated and chromatographed on silica gel to afford the guloside **10** (0.044 g; 0.08 mmol; 19 %): $[\alpha]_D$ – 28 (c 0.32, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38-7.21 (m, 15H, Ar), 4.86 (d, 1H, C*H*$_2$Ph, *J* = 11.9 Hz), 4.63 (d, 1H, H-1, $J_{1,2}$ = 7.9 Hz), 4.62 (d, 1H, C*H*$_2$Ph, *J* = 11.9 Hz), 4.56 (d, 1H, C*H*$_2$Ph, *J* = 12.0 Hz), 4.52 (d, 1H, C-*H*$_2$Ph, *J* = 11.9 Hz), 4.48 (d, 1H, C*H*$_2$Ph, *J* = 12.0 Hz), 4.47 (d, 1H, C*H*$_2$Ph, *J* = 11.9 Hz), 4.11 (ddd, 1H, H-5, $J_{5,4}$ = 1.3 Hz, $J_{5,6a}$ = $J_{5,6b}$ = 6.4 Hz), 4.06 (dd, 1H, H-3, $J_{3,2}$ = $J_{3,4}$ = 3.4 Hz), 3.68 (dd, 1H, H-6a, $J_{6a,6b}$ = 9.7 Hz), 3.64 (dd, 1H, H-6b), 3.59 (dd, 1H, H-4), 3.55 (dd, 1H, H-2), 3.54 (s, 3H, OC*H*$_3$), 2.48 (bs, 1H, O*H*-3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.3, 138.2, 138.0, 128.43, 128.33, 128.29, 127.99, 127.95, 127.85, 127.75, 127.62, 127.56, 101.5, 76.0, 75.7, 73.4, 73.3, 72.9, 71.8, 69.9, 68.2, 56.6; ESI HRMS calc'd for $C_{28}H_{32}O_6Na$: 487.2091. Found: 487.2091.

2-(Trimethylsilyl)ethyl 4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (11)

2-(Trimethylsilyl)ethyl 3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside[139] (1.456 g; 3.64 mmol) was mixed with acetonitrile (25 mL), benzaldehyde dimethyl acetal (1.60 mL; 10.66 mmol; 2.9 eq.) and *p*-toluenesulfonic acid (0.020 g). After stirring for 20 minutes triethylamine was added until the mixture was slightly basic and then solvents were evaporated. The residue was dissolved in dichloromethane (~ 20 mL) washed once with an equal volume of water, dried over sodium sulfate, filtered, and concentrated. Crystallization from ethyl acetate/hexanes gave **11** (1.451 g; 2.97 mmol; 82%): $[\alpha]_D$ 24 (c 0.49, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52-7.49 (m, 2H, Ar), 7.35-7.27 (m, 5H, Ar), 6.86-6.82 (m, 2H, Ar), 5.43 (s, 1H, C*H*Ph), 4.69 (d, 1H, C*H*$_2$Ar, *J* = 11.9 Hz), 4.65

(d, 1H, CH_2Ar, J = 11.9 Hz), 4.29 (dd, 1H, H-6a, $J_{6a,5}$ = 1.0 Hz, $J_{6a,6b}$ = 12.4 Hz), 4.28 (d, 1H, H-1, $J_{1,2}$ = 7.7 Hz), 4.09 (bd, 1H, H-4, $J_{4,3}$ = 3.3 Hz), 4.06-3.98 (m, 2H, H-a, H-6b), 3.94 (dd, 1H, H-2, $J_{2,3}$ = 9.5 Hz), 3.78 (s, 3H, OCH_3), 3.56 (ddd, 1H, H-a', J = 6.3 Hz, J = 9.6 Hz, J = 10.9 Hz), 3.45 (dd, 1H, H-3), 3.32 (s, 1H, H-5), 2.38 (s, 1H, OH-2), 1.07-0.96 (m, 2H, H-b, H-b'), 0.00 (s, 9H, Si$(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.2, 137.8, 130.1, 129.4, 128.8, 128.0, 126.3, 113.8, 102.4, 101.2, 78.8, 73.3, 71.1, 70.1, 69.3, 67.0, 66.7, 55.3, 18.3, -1.3; ESI HRMS calc'd for $C_{26}H_{36}O_7SiNa$: 511.2128. Found: 511.2127. Anal. calc'd for $C_{26}H_{36}O_7Si$: C 63.91, H 7.43. Found: C 63.88, H 7.66.

2-(Trimethylsilyl)ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (12)

2-(Trimethylsilyl)ethyl 3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside[139] (2.246 g; 5.6 mmol) was dissolved in acetonitrile (40.0 mL) and benzaldehyde dimethyl acetal (842 μL; 3.0 eq.). A catalytic amount of *p*-toluenesulfonic acid was added, and the mixture was stirred for 25 minutes and neutralized with triethylamine. Following concentration, the residue was dissolved in pyridine (25.0 ml) and benzoyl chloride (781 μL; 1.2 eq.). After stirring overnight, the mixture was concentrated, then redissolved in dichloromethane (100 mL) and washed with an equal volume of water. The organic extract was dried over sodium sulfate and concentrated. Recrystallization from ethyl acetate-hexanes yielded the title compound **12** (2.526 g; 4.3 mmol; 76%): $[\alpha]_D$ 33 (c 0.76, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03-7.99 (m, 2H, Ar), 7.58-7.52 (m, 3H, Ar), 7.45-7.41 (m, 2H, Ar), 7.38-7.31 (m, 3H, Ar), 7.14-7.10 (m, 2H, Ar), 6.70-6.66 (m, 2H, Ar), 5.57 (dd, 1H, H-2, $J_{2,1}$ = 8.0 Hz, $J_{2,3}$ = 9.9 Hz), 5.51 (s, 1H, CHPh), 4.62-4.48

(m, 3H, H-1, CH_2Ar), 4.33 (dd, 1H, H-6a, $J_{6a,5}$ = 1.3 Hz, $J_{6a,6b}$ = 12.2 Hz), 4.19 (d, 1H, H-4, $J_{4,3}$ = 3.5 Hz), 4.05 (dd, 1H, H-6b, $J_{6b,5}$ = 1.7 Hz), 3.98 (m, 1H, H-a), 3.73 (s, 3H, OCH_3), 3.69 (dd, 1H, H-3), 3.51 (m, 1H, H-a'), 3.39 (s, 1H, H-5), 0.84 (m, 2H, H-b, H-b'), -0.10 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.1, 159.2, 137.7, 132.7, 130.5, 129.9, 129.8, 129.3, 128.9, 128.2, 128.1, 126.5, 113.6, 101.2, 100.7, 76.6, 73.3, 70.9, 70.5, 69.2, 66.7, 66.5, 55.2, 17.9, -1.5; ESI HRMS calc'd for $C_{33}H_{40}O_8SiNa$: 615.2390. Found: 615.2391. Anal. calc'd for $C_{33}H_{40}O_8Si$: C 66.87, H 6.80. Found: C 66.55 H 6.83.

2-(Trimethylsilyl)ethyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (13)

2-(Trimethylsilyl)ethyl 3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside[139] (0.854 g; 2.13 mmol) was dissolved in acetonitrile (20.0 mL) and benzaldehyde dimethyl acetal (960 μL; 3.0 eq.). A catalytic amount of *p*-toluenesulfonic acid was added, and the mixture was stirred for 25 minutes then neutralized with triethylamine. After concentration and further evaporation from toluene, the residue was dissolved in *N,N*-dimethylformamide (10 mL) and sodium hydride (0.170 g of a 60% dispersion in oil; 4.25 mmol; 2 eq.) was added. After stirring for several minutes, benzyl bromide (0.303 mL; 2.56 mmol; 1.2 eq.) was added dropwise, and stirring was continued for a further 3 hours, then diluted with 1 mL of methanol. The reaction mixture was concentrated to roughly 1/3 of its initial volume, ethyl acetate (20 mL) was added, and the solids were removed by filtration. After concentration, the residue was recrystallized from ethanol to give the title compound **13** (0.877 g; 1.51 mmol; 71%): $[\alpha]_D$ 21 (c 1.23, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56-7.52 (m, 2H, Ar), 7.39-7.23 (m, 10H, Ar), 6.84-6.79 (m, 2H, Ar),

5.47 (s, 1H, C*H*Ph), 4.92 (d, 1H, Ar, J = 10.9 Hz), 4.76 (d, 1H, Ar, J = 10.9 Hz), 4.68 (m, 2H, Ar), 4.37 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 4.29 (dd, 1H, H-6a, $J_{6a,5}$ = 2.4 Hz, $J_{6a,6b}$ = 12.2 Hz), 4.07-3.96 (m, 3H, H-4, H-6b, H-a), 3.80 (dd, 1H, H-2, $J_{2,3}$ = 9.7 Hz), 3.78 (s, 3H, OCH_3), 3.56 (m, 1H, H-a'), 3.52 (dd, 1H, H-3, $J_{3,4}$ = 3.7 Hz), 3.28 (bs, 1H, H-5), 1.04 (m, 2H, H-b, H-b'), 0.01 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.2, 139.1, 137.2, 130.5, 129.3, 128.9, 128.2, 128.1, 128.0, 127.4, 126.6, 113.7, 103.2, 101.4, 78.8, 78.6, 75.2, 74.1, 71.6, 69.3, 67.3, 66.4, 55.2, 18.5, –1.4; ESI HRMS calc'd for $C_{33}H_{42}O_7SiNa$: 601.2598. Found: 601.2597. Anal. calc'd for $C_{33}H_{42}O_7Si$: C 68.48, H 7.31. Found: C 68.27. H 7.40.

2-(Trimethylsilyl)ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-β-D-galactopyranoside (14)

Galactopyranoside **12** (2.123 g; 3.6 mmol) was dissolved in a mixture of acetonitrile and water (16:1; 68 mL) and ceric ammonium nitrate (6.033 g; 11.0 mmol; 3.0 eq.) was added. After stirring for 30 minutes, the yellow solution was poured into dichloromethane (200 mL) and washed with water (2 x 150 mL). The organics were dried over sodium sulfate, filtered through cotton and concentrated. Chromatography on silica gel with hexanes/ethyl acetate (2:1) as eluent gave **14** (1.431 g; 85%). All spectral data were in accord with those reported for the synthesis of **14** by a different method.[140]

2-(Trimethylsilyl)ethyl 2-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (15)

Galactopyranoside **13** (0.137 g; 0.24 mmol) was dissolved in a mixture of acetonitrile and water (5 mL; 16:1), and then ceric ammonium nitrate (0.390 g; 0.71 mmol; 3 eq.) was added. After stirring for one hour, the mixture was diluted with dichloromethane (30 mL) and washed with water (2 x 20 mL), then dried over sodium sulfate, filtered, and

concentrated. Chromatography on silica gel with hexanes:ethyl acetate (2:1) as eluent afforded compound **15** (0.083 g; 0.18 mmol; 77%): $[\alpha]_D$ 5 (c 0.80, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53-7.47 (m, 2H, Ar), 7.39-7.24 (m, 8H, Ar), 5.54 (s, 1H, C*H*Ph), 4.98 (d, 1H, Ar, J = 11.3 Hz), 4.76 (d, 1H, Ar, J = 11.2 Hz), 4.40 (d, 1H, H-1, $J_{1,2}$ = 7.7 Hz), 4.32 (d, 1H, H-6a, $J_{6a,6b}$ = 12.4 Hz), 4.20 (d, 1H, H-4, $J_{4,3}$ = 3.8 Hz), 4.08-4.02 (m, 2H, H-6b, H-a), 3.72 (m, 1H, H-3), 3.62-3.55 (m, 2H, H-2, H-a'), 3.52 (bs, 1H, H-5), 1.04 (m, 2H, H-b, H-b'), 0.02 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (125 MHz, CDCl3) δ 138.6, 137.7, 129.0, 128.3, 128.1, 127.9, 127.5, 126.4, 103.1, 101.4, 79.4, 75.6, 74.8, 72.5, 69.2, 67.4, 66.5, 18.6, -1.3; ESI HRMS calc'd for $C_{25}H_{34}O_6SiNa$: 481.2017. Found: 481.2019. Anal. calc'd for $C_{25}H_{34}O_6Si$: C 65.47, H 7.47. Found: C 65.62. H 7.67.

2-(Trimethylsilyl)ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-gulopyranoside (16)

Alcohol **14** (0.780 g; 1.6 mmol) was dissolved in freshly distilled dichloromethane (15 mL) and pyridine (0.33 mL; 4.1 mmol; 2 eq.), then cooled to –20 °C with stirring under an argon atmosphere. Trifluoromethanesulfonic anhydride (0.450 mL; 2.6 mmol; 1.6 eq.) was added dropwise over a period of approximately one minute, and stirring was continued for 30 minutes. The reaction mixture was diluted with dichloromethane (50 mL), washed with water (2 x 50 mL), dried over sodium sulfate, filtered and concentrated. (^{1}H NMR data for crude triflate: (500 MHz, $CDCl_3$) δ 8.03-8.00 (m, 2H, Ar), 7.60-7.29 (m, 8H, Ar), 5.71 (dd, 1H, H-2, $J_{2,1}$ = 8.0 Hz, $J_{2,3}$ = 10.3 Hz), 5.59 (s, 1H, C*H*Ph), 5.10 (dd, 1H, H-3, $J_{3,4}$ = 3.7 Hz), 4.65 (d, 1H, H-1), 4.51 (bd, 1H, H-4), 4.39 (dd, 1H, H-6a, $J_{6a,5}$ = 1.5 Hz, $J_{6a,6b}$ = 12.6 Hz), 4.10 (dd, 1H, H-6b, $J_{6b,5}$ = 1.7 Hz), 4.00 (ddd, 1H, H-a, J = 5.5 Hz, J = 9.7 Hz, J = 10.7 Hz), 3.57-3.51 (m, 2H, H-a', H-5), 0.90-0.79 (m, 2H, H-b, H-b'), -0.11 (s, 9H, $Si(CH_3)_3$). The residue was dissolved in freshly

distilled acetonitrile (12 mL) and solid tetrabutylammonium nitrite (1.430 g; 4.95 mmol; 3 eq.) was added. After stirring overnight at room temperature, the solution was concentrated and the residue chromatographed using 3:1 hexanes/ethyl acetate as eluent to afford **16** (0.560 g; 1.2 mmol; 72%): $[\alpha]_D$ – 39 (c 0.18, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (m, 2H, Ar), 7.60-7.52 (m, 3H, Ar), 7.46-7.42 (m, 2H, Ar), 7.38-7.32 (m, 3H, Ar), 5.56 (s, 1H, C*H*Ph), 5.34 (dd, 1H, H-2, $J_{2,1}$ = 8.4 Hz, $J_{2,3}$ = 3.2 Hz), 5.05 (d, 1H, H-1), 4.39-4.34 (m, 2H, H-3, H-6a), 4.12-4.00 (m, 3H, H-6b, H-4, H-a), 3.87 (d, 1H, H-5, J = 1.3 Hz), 3.57 (ddd, 1H, H-a'), 2.24 (d, 1H, OH-3, $J_{OH3,3}$ = 2.4 Hz), 0.90 (m, 2H, H-b, H-b'), -0.09 ((s, 9H, $Si(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.0, 137.6, 133.2, 129.9, 129.8, 129.0, 128.4, 128.1, 126.4, 101.3, 97.4, 76.2, 71.4, 69.4, 69.3, 66.8, 65.6, 18.0 , -1.5; ESI HRMS calc'd for $C_{25}H_{32}O_7SiNa$: 495.1810. Found: 495.1811. Anal. calc'd for $C_{25}H_{32}O_7Si$: C 63.53, H 6.82. Found: C 63.31 H 6.82.

2-(Trimethylsilyl)ethyl 2-*O*-benzyl-4,6-*O*-benzylidene-β-D-gulopyranoside (17)

Alcohol **15** (0.150 g; 0.33 mmol) was dissolved in freshly distilled dichloromethane (8 mL) and pyridine (0.053 mL; 0.65 mmol; 2 eq.), then cooled to –20 °C with stirring under an argon atmosphere. Trifluoromethanesulfonic anhydride (0.078 mL; 0.46 mmol; 1.4 eq.) was added dropwise, and stirring was continued for 1 hour. The reaction mixture was then diluted with dichloromethane (30 mL), washed with water (2 x 15 mL), dried over sodium sulfate, filtered and concentrated. The residue was dissolved in freshly distilled acetonitrile (5 mL) and solid tetrabutylammonium nitrite (0.283 g; 0.98 mmol; 3 eq.) was added. No reaction was observed after stirring for one hour at room temperature, so the solution was heated to 50 °C. After stirring for a further two hours the solution was concentrated and the residue chromatographed using hexanes/ethyl

acetate (7:2) as eluent to afford **17** (0.060 g; 0.13 mmol; 40%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53-7.49 (m, 2H, Ar), 7.40-7.29 (m, 8H, Ar), 5.55 (s, 1H, C*H*Ph), 4.94 (d, 1H, CH_2Ar, J = 11.5 Hz), 4.81 (d, 1H, H-1, $J_{1,2}$ = 8.1 Hz), 4.67 (d, 1H, CH_2Ar), 4.35 (d, 1H, H-6a, $J_{6a,6b}$ = 12.6 Hz), 4.18 (dd, 1H, H-3, $J_{3,2} = J_{3,4}$ = 3.1 Hz), 4.12-4.04 (m, 3H, H-4, H-6b, H-a), 3.78 (bs, 1H, H-5), 3.66 (dd, 1H, H-2), 3.59 (m, 1H, H-a'), 2.62 (bs, 1H, OH-3), 1.05 (m, 2H, H-b, H-b'), 0.18 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4, 137.9, 129.1, 128.4, 128.2, 127.82, 127.79, 126.4, 101.3, 99.9, 75.99, 75.96, 73.5, 69.8, 69.4, 67.1, 65.4,18.5, -1.4; ESI HRMS calc'd for $C_{25}H_{34}O_6SiNa$: 481.2017. Found: 481.2017. Anal. calc'd for $C_{25}H_{34}O_6Si$: C 65.47, H 7.47. Found: C 65.33. H 7.89.

2-(Trimethylsilyl)ethyl 3-*S*-acetyl-2-*O*-benzoyl-4,6-*O*-benzylidene-3-thio-β-D-galactopyranoside (18)

Alcohol **16** (0.450 g; 0.95 mmol) was dissolved in dry dichloromethane and pyridine (0.385 mL; 4.76 mmol; 5.0 eq.), then cooled to –20 °C. Trifluoromethanesulfonic anhydride (0.640 mL; 3.80 mmol; 4.0 eq.) was added over approximately five minutes and the mixture was allowed to warm to room temperature. After 50 minutes dichloromethane (50 mL) was added, and the solution was washed with water (2 x 25 mL), dried over sodium sulfate, filtered, and concentrated. The residue was combined with potassium thioacetate (0.650 g; 5.80 mmol; 6.1 eq.), and *N,N*-dimethylformamide (10 mL) was added. The mixture was stirred overnight at 60 °C then diluted with ethyl acetate (75 mL), washed with water (2 x 50 mL), dried over sodium sulfate and concentrated. Chromatography on silica gel using hexanes:ethyl acetate (4.5:1→3.5:1) as eluent yielded **18** (0.398 g; 0.75 mmol; 79%): $[\alpha]_D$ 74 (c 0.32, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.98 (m, 2H, Ar), 7.55-7.50 (m, 3H, Ar), 7.43-7.32 (m, 5H, Ar), 5.54 (s,

1H, C*H*Ph), 5.42 (dd, 1H, H-2, $J_{2,1}$ = 7.8 Hz, $J_{2,3}$ = 11.5 Hz), 4.71 (d, 1H, H-1), 4.36 (dd, 1H, H-6a, $J_{6a,5}$ = 1.4 Hz, $J_{6a,6b}$ = 12.3 Hz), 4.22 (dd, 1H, H-3, $J_{3,4}$ = 3.3 Hz), 4.11 (dd, 1H, H-4, $J_{4,5}$ = 1.0 Hz), 4.08 (dd, 1H, H-6b, $J_{6b,5}$ = 1.7 Hz), 4.00 (1H, ddd, H-a), 3.78 (m, 1H, H-5), 3.53 (m, 1H, H-a'), 2.18 (s, 3H, SC(O)CH_3), 0.85 (m, 2H, H-b, H-b'), -0.12 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 195.0, 165.2, 137.4, 132.9, 129.87, 129.82, 129.0, 128.3, 128.1, 126.3, 101.9, 101.3, 75.9, 69.2, 69.0, 68.4, 66.8, 47.1, 30.5, 17.9, -1.5; ESI HRMS calc'd for $C_{27}H_{34}O_7SSi$ Na: 553.1692. Found: 553.1693. Anal. calc'd for $C_{27}H_{34}O_7SSi$: C 61.11, H 6.46. Found: C 61.55. H 6.33.

2-(Trimethylsilyl)ethyl 3-thio-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside disulfide (20)

The thioester **18** (0.100 g; 0.18 mmol) was dissolved in *N,N*-dimethylformamide (5 mL) and argon was bubbled through the solution for twenty minutes before the addition of hydrazine acetate (0.035 g; 0.38 mmol; 2.0 eq.). After 5 hours, the reaction mixture was diluted with ethyl acetate (40 mL) and washed with water (2x30 mL), then dried over sodium sulfate. The ^{1}NMR spectrum of the crude product **19** was contaminated with disulfide **20**. ^{1}H NMR data for the crude thiol (400 MHz, $CDCl_3$) δ 8.14-8.10 (m, 2H, Ar), 7.63-7.59 (m, 1H, Ar), 7.52-7.45 (m, 4H, Ar), 7.31-7.28 (m, 3H, Ar), 5.59 (s, 1H, C*H*Ph), 5.31 (dd, 1H, H-2, $J_{2,1}$ = 7.7 Hz, $J_{2,3}$ = 11.6 Hz), 4.59 (d, 1H, H-1), 4.35 (dd, 1H, H-6a, $J_{6a,5}$ = 12.3 Hz, $J_{6a,6b}$ = 12.3 Hz), 4.14 (bd, 1H, H-4, $J_{4,3}$ = 3.2 Hz), 4.08 (dd, 1H, H-6b, $J_{6b,5}$ = 1.6 Hz), 4.00 (ddd, 1H, H-a, J = 5.5 Hz, J = 8.6 Hz, J = 10.7 Hz), 3.6 (bs, 1H, H-5), 3.54 (ddd, 1H, H-a', J = 6.7 Hz, J = 9.7 Hz, J = 10.7 Hz), 3.12 (ddd, 1H, H-3, $J_{3,SH}$ = 11.0 Hz), 2.20 (d, 1H, SH), 0.89 (ddd, 1H, H-b, J = 6.6 Hz, J = 10.5 Hz, J = 13.9 Hz), (ddd, 1H, H-b', J = 5.6 Hz, J = 10.2 Hz, J = 13.9 Hz), -0.10 (s, 9H, $Si(CH_3)_3$). The

crude mixture was dissolved in tetrahydrofuran (4 mL), and sodium hydride (0.0075 g of a 60% dispersion in oil; 0.18 mmol; 1.0 eq.) was added. After stirring overnight, the mixture was diluted with dichloromethane (25 mL), washed with brine then water, dried over sodium sulfate, and concentrated. Chromatography on silica gel with hexanes/ethyl acetate (5:2) as eluent yielded **20** (0.092 g; 0.09 mmol; 92%): $[\alpha]_D$ 107 (c 1.36, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14-8.10 (m, 2H, Ar), 7.63-7.59 (m, 1H, Ar), 7.52-7.45 (m, 4H, Ar), 7.31-7.28 (m, 3H, Ar), 5.45 (s, 1H, C*H*Ph), 5.27 (dd, 1H, H-2, $J_{2,1}$ = 7.7 Hz, $J_{2,3}$ = 11.6 Hz), 4.27 (d, 1H, H-1), 4.18 (dd, 1H, H-6a, $J_{6a,5}$ = 12.3 Hz, $J_{6a,6b}$ = 12.3 Hz), 4.13 (bd, 1H, H-4, $J_{4,3}$ = 3.2 Hz), 3.91 (ddd, 1H, H-a, J = 5.5 Hz, J = 8.6 Hz, J = 10.7 Hz), 3.86 (dd, 1H, H-6b, $J_{6b,5}$ = 1.6 Hz), 3.44 (ddd, 1H, H-a', J = 6.7 Hz, J = 9.7 Hz, J = 10.7 Hz), 3.09 (dd, 1H, H-3), 2.89 (bs, 1H, H-5), 0.86-0.74 (m, 2H, H-b, H-b'), -0.11 (s, 9H, Si(CH3)3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.9, 137.4, 133.2, 130.3, 129.9, 129.8, 128.5, 128.0, 126.2, 101.4, 101.0, 76.6, 74.6, 70.2, 68.9, 68.5, 66.6, 58.5, 17.9, -1.5; ESI HRMS calc'd for $C_{50}H_{62}O_{12}S_2SiNa$: 997.311898. Found: 997.311601; Anal. calc'd for $C_{50}H_{62}O_{12}S_2Si$: C 61.57, H 6.41. Found: C 61.28 , H 6.62.

Allyl 2,3-di-*O*-acetyl-4,6-*O*-isopropylidene-α/β-D-gulopyranoside (21)

1,2,3-Tri-*O*-acetyl-4,6-*O*-isopropylidene-α/β-gulopyranose[144] (0.102 g; 0.29 mmol) was dissolved in *N,N*-dimethylformamide (4.0 mL) and hydrazine acetate (0.041 g; 0.44 mmol; 1.5 eq.) was added. After stirring for several hours, the mixture was diluted with ethyl acetate and washed with water, then dried over sodium sulfate, filtered and concentrated. ^{1}H NMR data for crude 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-gulopyranose (600 MHz, $CDCl_3$) δ 5.28 (dd, 1H, H-3, $J_{3,2} \sim J_{3,4} \sim$ 3.3 Hz), 5.08 (dd, 1H, H-2, $J_{2,1}$ = 8.4 Hz), 4.97 (d, 1H, H-1), 4.07 (dd, 1H, H-6a, $J_{6a,5}$ = 2.1 Hz, $J_{6a,6b}$ = 13.0

Hz), 3.97 (dd, 1H, H-6b, $J_{6b,5}$ = 1.6 Hz), 3.94 (ddd, 1H, H-4, $J_{4,5}$ = 1.3 Hz), 3.78 (bs, 1H, H-5), 2.14 (s, 3H, $OC(O)CH_3$), 2.08 (s, 3H, $OC(O)CH_3$), 1.46, 1.44 (2s, 6H, $C(CH_3)_2$). The residue was dissolved in a mixture of tetrahydrofuran and *N,N*-dimethylformamide (1:1; 4.0 mL) and sodium hydride (11.0 mg of a 60% dispersion in oil; 1.5 eq.) was added, followed by allyl bromide (0.052 mL; 0.57 mmol; 2 eq.). After approximately one hour, the solution was neutralized with acetic acid and concentrated. Chromatography of the residue on silica gel afforded **21** (3:1 β/α) (0.068 g; 0.24 mmol; 67%). Data for the β-anomer: $[\alpha]_D$ –85 (c 0.54, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.89 (dddd, 1H, H-b, *J* = 5.0 Hz, *J* = 5.7 Hz, *J* = 10.8 Hz, *J* = 17.3 Hz), 5.33 (dd, 1H, H-3, $J_{3,2} \sim J_{3,4} \sim$ 3.4 Hz), 5.29 (dddd, 1H, H-c, *J* = 1.7 Hz, *J* =1.7 Hz, *J* = 1.7 Hz, J = 17.3 Hz), (dddd, 1H, H-d, *J* = 1.7 Hz, *J* = 1.7 Hz, *J* = 1.7 Hz, *J* = 10.8 Hz), 5.14 (dd, 1H, H-2, $J_{2,1}$ = 8.4 Hz), 4.80 (d, 1H, H-1), 4.38 (dddd, 1H, H-a, *J* = 1.5 Hz, *J* = 1.5 Hz, *J* = 5.0 Hz, *J* = 13.3 Hz), 4.10 (dddd, 1H, H-a', *J* = 1.5 Hz, *J* = 1.5 Hz, *J* = 5.9 Hz, *J* = 13.3 Hz), 4.05 (dd, 1H, H-6a, $J_{6a,5}$ = 2.3 Hz, $J_{6a,6b}$ = 12.9 Hz), 3.95 (dd, 1H, H-6b, $J_{6b,5}$ = 12.9 Hz), 3.90 (dd, 1H, H-4, $J_{4,5}$ = 1.3 Hz), 3.62-3.60 (m, 1H, H-5), 2.12, 2.02 (2s, 6H, 2x$C(O)CH_3$), 1.46, 1.42 (2s, 6H, $C(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.34, 169.32, 134.1, 116.8, 98.9, 97.1, 69.9, 69.3, 68.1, 67.7, 65.9, 62.4, 29.1, 20.82, 20.79, 18.6; ESI HRMS calc'd for $C_{16}H_{24}O_8Na$: 367.1363. Found: 367.1363; ^{1}H NMR data for the α-anomer: (500 MHz, $CDCl_3$) δ 5.91 (dddd, 1H, H-b, *J* = 4.8 Hz, *J* = 5.7 Hz, *J* = 10.4 Hz, *J* = 17.2 Hz), 5.35 (H-c, *J* = 1.8 Hz, *J* = 1.9 Hz, *J* = 1.9 Hz, *J* = 17.2 Hz), 5.25 (dd, 1H, H-2, $J \sim J \sim$ 4.0 Hz), 5.21-5.17 (m, 2H, H-4, H-d), 5.07 (d, 1H, H-1, $J_{1,2}$ = 4.0 Hz), 4.24 (dddd, H-a, *J* = 1.9 Hz, *J* = 1.9 Hz, *J* = 4.7 Hz, *J* = 13.5 Hz), 4.08-4.02 (m, 2H, H-6a, H-a'), 3.98 (dd, 1H, H-4, *J* = 1.4 Hz, *J* = 3.3 Hz), 3.89 (dd, 1H, H-6b, $J_{6b,5}$ = 1.9 Hz, $J_{6b,6a}$ = 12.8 Hz),

3.83 (m, 1H, H-5), 2.13 (s, 3H, C(O)CH_3), 2.07 (s, 3H, C(O)CH_3), 1.45, 1.44 (2s, 6H, C(CH_3)$_2$).

Allyl 2,3,4,6-tetra-*O*-acetyl-β-D-gulopyranoside (22)

1,2,3,4,6-Penta-*O*-acetyl-β-D-gulopyranose[278] (20.0 g; 51.2 mmol) was evaporated from toluene (150 mL) and concentrated. The syrup was dissolved in dry dichloromethane (60 mL) and cooled to 0 °C before adding HBr in acetic acid (45% (w/v); 60 mL). After allowing the mixture to warm to RT over 2 hours, the solution was evaporated to dryness and then concentrated from toluene (3x60 mL). The yellow residue was then dissolved in dichloromethane (250 mL) and washed with equal volumes of saturated sodium bicarbonate solution followed by water. The organic layers were dried over sodium sulfate, filtered, and concentrated. The syrup obtained was dissolved in allyl alcohol (150 mL) and Drierite (20 g) was added. After stirring the mixture for 30 mins., mercury (II) cyanide (7.0 g; 27.7 mmol; 0.54 eq.) and mercury (II) bromide (0.74 g; 2.1 mmol; 0.04 eq.) were added. The reaction mixture was stirred at room temperature under Argon overnight and then concentrated. The residue was suspended in dichloromethane (250 mL), filtered, and washed with 10% potassium bromide solution and water, then dried over sodium sulfate, filtered and concentrated. Column chromatography using a gradient of hexanes and ethyl acetate (3:1 → 5:2) afforded **22** (14.76 g; 38.0 mmol; 74%): $[\alpha]_D$ – 29 (c 1.21, CHCl3); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.90 (dddd, 1H, H-b, J = 5.0 Hz, J = 6.0 Hz, J = 10.5 Hz, J = 17.2 Hz), 5.41 (dd, 1H, H-3, $J_{3,2}$ = $J_{3,4}$ = 3.7 Hz), 5.30 (dddd, 1H, H-c, J =1.6 Hz, J = 1.6 Hz, J = 1.6 Hz, J = 17.2 Hz), 5.21 (dddd, 1H, H-d, J = 1.4 Hz, J = 1.4 Hz, J = 1.4 Hz, J = 10.5 Hz), 5.05 (dd, 1H, H-2, $J_{2,1}$ = 8.0 Hz), 4.98 (dd, 1H, H-4, $J_{4,5}$ = 1.7 Hz), 4.81 (d, 1H, H-1), 4.36 (dddd, 1H, H-a, J = 1.5 Hz, J = 1.5 Hz, J = 5.0 Hz, J =

13.1 Hz), 4.19-4.16 (m, 3H, H-5, H-6a, H-6b), 4.13 (ddd, 1H, H-a', J = 1.4 Hz, J = 1.4 Hz, J = 6.0 Hz, J = 13.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 170.5, 170.3, 169.8, 134.3, 118.1, 98.0, 70.8, 70.2, 68.8, 68.1, 68.0, 62.2, 20.84, 20.81, 20.79, 20.7; ESI HRMS calc'd for $C_{17}H_{24}O_{10}Na$: 411.1261. Found: 411.1262. Anal. calc'd for $C_{17}H_{24}O_{10}$: C 52.57, H 6.23. Found: C 52.96, H 6.14.

Allyl β-D-gulopyranoside (23)

The tetraacetate **22** (1.488 g; 3.9 mmol) was suspended in anhydrous methanol (10 mL) under a stream of argon. A small piece of sodium metal was added, and the reaction mixture was stirred overnight. The solution was then made neutral by addition of acidic ion exchange resin, and the solids were removed by filtration. A colourless solid was obtained after evaporation of the solvents (0.825 g; 3.7 mmol; 98%). A small portion of this material was further purified for characterization as follows. Silica gel (2 g) was added to a solution of the tetrol (~100mg) in methanol and the suspension was evaporated to dryness. The solid was applied to the top of a silica gel column, which was then eluted with ethyl acetate-methanol (12:1) to afford pure **23** (87 mg): $[\alpha]_D$ –58 (c 0.27, MeOH). ^{1}H NMR (600 MHz, CD_3OD, δ) 5.96 (dddd, 1H, H-b, J = 5.3 Hz, J = 6.1 Hz, J = 10.6 Hz, J = 17.2 Hz), 5.31 (dddd, 1H, H-c, J = 1.8 Hz, J = 1.8 Hz, J = 1.8 Hz, J = 17.2 Hz), 5.14 (dddd, H-d, J = 1.4 Hz, J = 1.4 Hz, J = 1.8 Hz, J = 10.5 Hz), 4.63 (d, 1H, H-1, $J_{1,2}$ = 8.2 Hz), 4.36 (dddd, 1H, H-a, J = 1.6 Hz, J = 1.6 Hz, J = 5.2 Hz, J = 12.9 Hz), (dddd, 1H, H-a', J = 1.4 Hz, J = 1.4 Hz, J = 6.0 Hz, J = 12.9 Hz), 3.94 (dd, 1H, H-3, $J_{3,2} = J_{3,4}$ = 3.6 Hz), 3.88-3.86 (m, 1H, H-5), 3.74 (dd, 1H, H-6a, $J_{6a,5}$ = 6.8 Hz, $J_{6a,6b}$ = 11.5 Hz), 3.71-3.67 (m, 2H, H-4, H-6b), 3.63 (dd, 1H, H-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.0, 117.2, 101.5, 75.0, 73.2, 71.3, 70.8, 69.7, 62.7; ESI HRMS calc'd for

$C_9H_{16}O_6Na$: 243.0839. Found: 243.0838.

Allyl (*S*)-4,6-*O*-benzylidene-β-D-gulopyranoside (24)

The guloside **23** (0.170 g; 0.77 mmol) was combined with *N,N*-dimethylformamide (3.0 mL) and acetonitrile (3.0 mL). Benzaldehyde dimethyl acetal (0.127 mL; 0.85 mmol; 1.1 eq.) was added, followed by *p*-toluenesulfonic acid monohydrate (6 mg), and the mixture was heated at 50°C overnight. The reaction mixture was made basic by addition of triethylamine, then concentrated. The residue was dissolved in dichloromethane and acetone, mixed with silica gel (1 g), and concentrated. The residue was applied to a silica gel column and eluted with a linear gradient of hexanes and ethyl acetate (2:1→1:1) to afford **24** (0.175 g; 0.56 mmol; 73%): $[\alpha]_D$ –81 (c 1.06, CHCl3); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53-7.48 (m, 2H, Ar), 7.39-7.31 (m, 3H, Ar), 5.96 (ddd, 1H, H-b, J = 5.3 Hz, J = 6.5 Hz, J = 10.4 Hz, J = 17.0 Hz), 5.32 (dddd, 1H, H-c, J = 1.6 Hz, J = 1.6 Hz, J = 1.6 Hz, J = 17.1 Hz), 5.25-5.21 (m, 1H, H-d), 4.75 (d, 1H, H-1, $J_{1,2}$ = 8.2 Hz), 4.46 (m, 1H, H-a), 4.35 (dd, 1H, H-6a, $J_{6a,5}$ = 1.1 Hz, $J_{6a,6b}$ = 12.4 Hz), 4.21 (dd, 1H, H-3, $J_{3,2} = J_{3,4}$ = 3.2 Hz), 4.15-4.06 (m, 3H, H-a', H-4, H-6b), 3.87 (dd, 1H, H-2), 3.79 (bs, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.7, 134.0, 129.1, 128.2, 126.3, 117.9, 101.3, 99.1, 75.9, 70.0, 69.9, 69.3, 68.9, 66.0; ESI HRMS calc'd for $C_{16}H_{20}O_6Na$: 331.1152. Found: 331.1155. Anal. calc'd for $C_{16}H_{20}O_6$: C 62.33, H 6.54. Found: C 62.14, H 6.65.

A small amount of the product was acetylated to identify the position of the free hydroxyl groups, yielding allyl 2,3-di-*O*-acetyl-(*S*)-4,6-*O*-benzylidene-β-D-gulopyranoside: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54-7.50 (m, 2H, Ar), 7.40-7.34 (m, 3H, Ar), 5.91 (dddd, 1H, H-b, J = 4.9 Hz, J = 5.8 Hz, J = 10.5 Hz, J = 17.3 Hz), 5.54 (s, 1H, C*H*Ph), 5.49 (dd, 1H, H-3, $J_{3,2} = J_{3,4}$ = 3.4 Hz), 5.30 (dddd, 1H, H-c, J = 1.7 Hz, J = 1.7

Hz, J = 1.7 Hz, J = 17.3 Hz), 5.21 (dd, 1H, H-2, $J_{2,1}$ = 8.6 Hz), 5.21-5.16 (m, 1H, H-d), 4.88 (d, 1H, H-1), 4.40 (dddd, 1H, H-a, J = J = 1.6 Hz, J = 1.6 Hz, J = 5.0 Hz, J = 13.2 Hz), 4.35 (dd, 1H, H6a, $J_{6a,5}$ = 1.5 Hz, $J_{6a,6b}$ = 12.6 Hz), 4.13 (dddd, 1H, H-a', J = 1.4 Hz, J = 1.4 Hz, J = 5.9 Hz, J = 13.2 Hz), 4.08 (dd, 1H, H-6b, $J_{6b,5}$ = 1.9 Hz), 4.01 (dd, 1H, H-4, $J_{4,5}$ = 1.3 Hz), 3.75 (m, 1H, H-5), 2.15 (s, 3H, C(O)CH_3), 2.03 (s, 3H, C(O)CH_3).

When the initial reaction was performed under identical conditions using *N,N*-dimethylformamide or acetonitrile as the lone solvent, slightly lower yields were realized, and a second isomer (~5%), identified as **allyl (*R*)-4,6-*O*-benzylidene-β-D-gulopyranoside (25)** could be recrystallized (ethyl acetate-hexanes) after chromatography as described above: $[\alpha]_D$ –81 (c 0.89, CHCl3); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50-7.46 (m, 2H, Ar), 7.44-7.39 (m, 2H, Ar), 7.37-7.34 (m, 1H, Ar), 6.14 (s, 1H, C*H*Ph), 5.97 (dddd, 1H, H-b, J = 5.6 Hz, J = 6.4 Hz, J = 10.4 Hz, J = 16.9 Hz), 5.33 (dddd, 1H, H-c, J = 1.6 Hz, J = 1.6 Hz, J = 1.6 Hz, J = 17.1 Hz), 5.24 (dddd, 1H, H-d, J = 10.3 Hz, 1.3 Hz, 1.3 Hz, 1.3 Hz), 4.71 (d, 1H, H-1, $J_{1,2}$ = 7.7 Hz), 4.46 (m, 1H, H-a), 4.26 (dd, 1H, H-3, $J_{3,2}$ = $J_{3,4}$ = 2.5 Hz), 4.12 (m, 1H, H-a'), 4.06-4.01 (m, 3H, H-4, H-6_a, H-6_b), 3.95 (dd, 1H, H-2), 3.78 (m, 1H, H-5), 2.55 (bs, 1H, O*H*), 2.49 (bs, 1H, O*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.7, 134.0, 129.1, 128.2, 126.3, 117.9, 101.3, 99.1, 75.9, 70.0, 69.9, 69.3, 68.9, 66.0; ESI HRMS calc'd for $C_{16}H_{20}O_6Na$: 331.1152. Found: 331.1154. Anal. calc'd for $C_{16}H_{20}O_6$: C 62.33, H 6.54. Found: C 62.09, H 6.51.

A small amount of the product was acetylated to identify the position of the free hydroxyl groups, yielding allyl 2,3-di-*O*-acetyl-(*R*)-4,6-*O*-benzylidene-β-D-gulopyranoside: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47-7.44 (m, 2H, Ar), 7.39-7.32 (m, 3H, Ar), 5.99 (s, 1H, C*H*Ph), 5.95-5.87 (m, 2H, H-3, H-b), 5.33 (dddd, 1H, H-c, J = 1.6 Hz, J

= 1.6 Hz, J = 1.6 Hz, J = 17.4 Hz), 5.30 (dd, 1H, H-2, $J_{2,3}$ = 3.4 Hz, $J_{2,1}$ = 4.4 Hz), 5.24 (dddd, 1H, H-d, J = 1.4 Hz, J = 1.4 Hz, J = 1.4 Hz, J = 10.5 Hz), 4.82 (d, 1H, H-1, $J_{1,2}$ = 4.5 Hz), 4.34-4.24 (m, 3H, H-4, OCH_2CHCH_2, H-6a), 4.19 (m, 1H, H-5), 4.12-4.04 (m, 2H, H-6b, OCH_2CHCH_2), 2.12, 2.08 (2s, 6H, $2xC(O)CH_3$).

Allyl 2-*O*-benzoyl-(*S*)-4,6-*O*-benzylidene-β-D-gulopyranoside (26) and **Allyl 2,3-di-*O*-benzoyl-(*S*)-4,6-*O*-benzylidene-β-D-gulopyranoside (27)**

Diol **24** (4.00 g; 13.0 mmol) was dissolved in a mixture of dry dichloromethane and pyridine (4:1; 60 mL), and the flask was immersed in an ice bath. Benzoyl chloride (1.66 mL; 14.3 mmol; 1.1 eq.) was added, and after 40 minutes the reaction mixture was quenched by addition of methanol (5 mL). After stirring for 20 minutes at room temperature the solution was concentrated. The residue was dissolved in dichloromethane (100 mL), washed successively with 1M HCl, saturated sodium bicarbonate, and water, then dried over sodium sulfate, filtered and concentrated. Column chromatography in hexanes/ethyl acetate (2:1) yielded **26** (5.01 g; 12.1 mmol; 94%): $[\alpha]_D$ –40 (c 0.76, CHCl3); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.07-8.04 (m, 2H, Ar), 7.62-7.58 (m, 1H, Ar), 7.57-7.54 (m, 2H, Ar), 7.49-7.45 (m, 2H, Ar), 7.41-7.35 (m, 3H, Ar), 5.85 (dddd, 1H, H-b, J = 5.0 Hz, J = 6.1 Hz, J = 10.6 Hz, J = 17 Hz), 5.59 (s, 1H, C*H*Ph), 5.41 (dd, 1H, H-2, $J_{2,1}$ = 8.5 Hz, $J_{2,3}$ = 3.3 Hz), 5.26 (dddd, 1H, H-c, J = 1.6 Hz, J = 1.6 Hz, J = 1.6 Hz, J = 17.2 Hz), 5.13 (dddd, 1H, H-d, J = 1.4 Hz, J = 1.4 Hz, J = 1.4 Hz, J = 10.6 Hz), 5.11 (d, 1H, H-1), 4.44-4.36 (m, 2H, H-a, H-6a), 4.19-4.10 (m, 2H, H-a', H-6b), 3.89 (m, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.1, 137.6, 134.1, 133.3, 129.82, 129.77, 129.1, 128.5, 128.2, 126.4, 117.1, 101.3, 96.9, 76.3, 71.3, 69.5, 69.28, 69.27, 65.7; ESI HRMS calc'd for $C_{23}H_{24}O_7Na$: 435.1414. Found: 435.1414. Anal.

calc'd for $C_{23}H_{24}O_7$: C 66.98, H 5.87. Found: C 67.06, H 5.79. A second, less polar compound **27** was also isolated (0.064 g; 0.12 mmol; 1 %): $[\alpha]_D$ – 88 (c 0.35, CHCl3); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08-8.05 (m, 2H, Ar), 7.92-7.87 (m, 2H, Ar), 7.67-7.62 (m, 1H, Ar), 7.60-7.56 (m, 2H, Ar), 7.53-7.47 (m, 3H, Ar), 7.43-7.36 (m, 3H, Ar), 7.35-7.29 (m, 2H, Ar), 5.93-5.83 (m, 2H, H-3, H-b), 5.63 (dd, H-2, $J_{2,1}$ = 8.4 Hz, $J_{2,3}$ = 3.5 Hz), 5.62 (s, 1H, C*H*Ph), 5.29 (dddd, 1H, H-c, J = 1.7 Hz, J = 1.7 Hz, J = 1.7 Hz, J = 17.2 Hz), 5.23 (d, 1H, H-1), 5.14 (dddd, 1H, H-d, J = 1.4 Hz, J = 1.4 Hz, J = 1.4 Hz, J = 10.6 Hz), 4.49-4.39 (m, 2H, H-a, H-6a), 4.26-4.19 (m, 2H, H-4, H-a'), 4.14 (m, 1H, H-6b), 3.93 (m, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.93, 164.91, 137.3, 134.0, 133.6, 133.0, 129.8, 129.71, 129.69, 129.4, 129.2, 128.7, 128.25, 128.22, 126.4, 117.0, 101.3, 97.7, 74.3, 70.5, 69.4, 69.1, 68.6, 66.3; ESI HRMS calc'd for $C_{30}H_{28}O_8Na$: 539.1676. Found: 539.1679. Anal. calc'd for $C_{30}H_{28}O_8$: C 69.76, H 5.46. Found: C 69.66, H 5.65.

Allyl 3-*S*-acetyl-2-*O*-benzoyl-(S)-4,6-*O*-benzylidene-3-thio-β-D-galactopyranoside (28)

Alcohol **26** (2.20 g; 5.3 mmol) was dissolved in dichloromethane (40 mL) containing pyridine (2.10 mL; 27 mmol; 5 eq.). The solution was cooled to –20 °C and trifluoromethanesulfonic anhydride (1.20 mL; 7.1 mmol; 1.34 eq.) was added over a period of one minute. The mixture was allowed to warm to –5 °C over 1.5 hours, and was then stirred at room temperature for a further 1.5 hours. After diluting with dichloromethane (40 mL), the reaction mixture was washed with an equal volume of water, dried over sodium sulfate, filtered, and concentrated. The residue was dissolved in *N*,*N*-dimethylformamide (50 mL), potassium thioacetate (1.30 g; 11.4 mmol, 2 eq.)

was added, and mixture was immersed in an oil bath at 50 °C. After stirring overnight, the black solution was concentrated. The syrup was dissolved in dichloromethane (50 mL) and washed with an equal volume of water, dried over sodium sulfate, filtered and concentrated. The yellow residue was chromatographed on a silica gel column (3.5 cm x 30 cm) capped by a 2 cm deep plug of decolourising carbon using hexanes-ethyl acetate (2:1) as eluent to yield **28** (2.36 g; 5.0 mmol; 94%): $[\alpha]_D$ 156 (c 0.48, CHCl3); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.01-7.99 (m, 2H, Ar), 7.58-7.52 (m, 3H, Ar), 7.46-7.42 (m, 2H, Ar), 7.41-7.36 (m, 3H, Ar), 5.77 (dddd, 1H, H-b, J = 4.8 Hz, J = 6.2 Hz, J = 10.8 Hz, J = 17.2 Hz), 5.56 (s, 1H, C*H*Ph), 5.49 (dd, 1H, H-2, $J_{2,1}$ = 7.7 Hz, $J_{2,3}$ = 11.5 Hz), 5.20 (dddd, 1H, H-c, J = 1.7 Hz, J = 1.7 Hz, J = 1.7 Hz, J = 17.2 Hz), 5.09 (dddd, 1H, H-d, J = 1.4 Hz, J = 1.4 Hz, J = 1.4 Hz, J = 10.8 Hz), 4.78 (d, 1H, H-1), 4.39-4.35 (m, 2H, H-6a, H-a), 4.25 (dd, 1H, H-3, $J_{3,4}$ = 3.3 Hz), 4.17-4.07 (m, 3H, H-4, H-6b, H-a'), 3.70 (m, 1H, H-5), 2.21 (s, 3H, C(O)CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 194.9, 165.2, 137.4, 133.8, 133.0, 129.8, 129.7, 129.0, 128.3, 128.2, 126.3, 117.2, 101.3, 101.1, 75.9, 69.3, 69.04, 69.01, 68.5, 47.0, 30.5; ESI HRMS calc'd for $C_{25}H_{26}O_7SNa$: 493.129695. Found: 493.129366. Anal. calc'd for $C_{25}H_{26}O_7S$: C 63.81, H 5.57. Found: C 63.95, H 5.51.

Allyl 2-*O*-benzoyl-(*S*)-4,6-*O*-benzylidene-3-thio-β-D-galactopyranoside (29)

Thioester **28** (0.320 g; 0.68 mmol) was dissolved in *N*,*N*-dimethylformamide (5.0 mL), and argon was bubbled through the solution for twenty minutes before hydrazine acetate (0.113 g; 1.23 mmol; 1.7 eq.) was added. The mixture was stirred for two hours, diluted with ethyl acetate (50 mL), washed with water (2x40 mL), and the organic layers dried over sodium sulfate, filtered and concentrated to afford **29** (0.286 g; 0.67 mmol; 98%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09-8.05 (m, 2H, Ar), 7.59-7.55 (m, 3H, Ar), 7.49-7.37

(m, 5H, Ar), 5.79 (dddd, 1H, H-b, J = 4.8 Hz, J = 6.2 Hz, J = 11.0 Hz, J = 17.3 Hz), 5.61 (s, 1H, C*H*Ph), 5.37 (dd, 1H, H-2, $J_{2,1}$ = 7.8 Hz, $J_{2,3}$ = 11.2 Hz), 5.21 (dddd, 1H, H-c, J = 1.7 Hz, J = 1.7 Hz, J = 1.7 Hz, J = 17.3 Hz), 5.09 (dddd, 1H, H-d, J = 1.4 Hz, J = 1.4 Hz, J = 1.4 Hz, J = 10.5 Hz), 4.66 (d, 1H, H-1), 4.38 (dd, 1H, H-6a, $J_{6a,6b}$ = 12.4 Hz$J_{6a,5}$ = 1.5 Hz), 4.39-4.33 (m, 1H, H-a), 4.17-4.09 (m, 3H, H-4, H-a', H-6b), 3.61 (m, 1H, H-5), 3.14 (ddd, 1H, H-3, $J_{3,4}$ = 3.2 Hz, $J_{3,2}$ = 11.0 Hz, $J_{3,SH}$ = 11.0 Hz), 2.24 (d, 1H, S*H*); ^{13}C NMR, (125 MHz, $CDCl_3$) δ 165.4, 137.5, 133.9, 133.0, 130.1, 129.8, 129.1, 128.3, 128.2, 128.3, 117.2, 101.5, 101.0, 76.6, 72.5, 69.2, 69.03, 68.96, 43.8; ESI HRMS calc'd for $C_{23}H_{24}O_6SNa$: 451.1191. Found: 451.1191. The title compound was slowly converted to allyl 2-*O*-benzoyl-(*S*)-4,6-*O*-benzylidene-3-thio-β-D-galactopyranoside disulfide in solution: ^{1}H NMR (500 MHz, $CDCl_3$, δ 8.09-8.06 (m, 2H, Ar), 7.60-7.53 (m, 3H, Ar), 7.47-7.43 (m, 2H, Ar), 7.40-7.34 (m, 3H, Ar), 5.78 (dddd, 1H, H-b, J = 4.8 Hz, J = 6.1 Hz, J = 10.6 Hz, J = 17.2 Hz), 5.60 (s, 1H, C*H*Ph), 5.59 (dd, 1H, H-2, $J_{2,1}$ = 7.8 Hz, $J_{2,3}$ = 11.2 Hz), 5.20 (dddd, 1H, H-c, J = 1.6 Hz, J = 1.6 Hz, J = 1.6 Hz, J = 17.2 Hz), 5.08 (dddd, 1H, H-d, J = 1.6 Hz, J = 1.6 Hz, J = 1.6 Hz, J = 10.6 Hz), 4.71 (d, 1H, H-1), 4.39-4.34 (m, 2H, H-6a, H-a), 4.33 (dd, 1H, H-4, J = 0.9 Hz, J = 3.2 Hz), 4.16-4.09 (m, 2H, H-6b, H-a'), 3.56 (bs, 1H, H-5), 2.99 (dd, 1H, H-3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.2, 137.6, 133.9, 133.0, 130.1, 129.8, 128.9, 128.4, 128.1, 126.3, 117.1, 101.4, 101.2, 78.4, 69.9, 69.2, 69.1, 68.9, 49.3; ESI HRMS calc'd for $C_{46}H_{46}O_{12}S_2Na$: 877.2328. Found: 877.2327. Anal. calc'd for $C_{46}H_{46}O_{12}S_2$: C 62.93, H 5.28. Found: C 63.18. H 5.40.

2-(Trimethylsilyl)ethyl *S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonate)-(2→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-3-thio-β-D-galactopyranoside (31)

Thioacetate **19** (0.253 g; 0.48 mmol) was dissolved in degassed *N,N*-dimethylformamide and hydrazine acetate (0.066 g; 0.72 mmol; 1.5 eq.) was added. After stirring under an argon atmosphere for 90 minutes, the reaction mixture was diluted with ethyl acetate (80 mL) and washed with water (2 x 40 mL). After drying over sodium sulfate, the organics were filtered and concentrated. The residue was dissolved in a small volume of dichloromethane and passed through a short pad of silica gel. Concentration of the eluent yielded a colourless solid (0.224 g) which was dissolved in degassed *N,N*-dimethylformamide (6.0 mL). Solid sodium hydride (0.021 g of a 60% dispersion in mineral oil; 1.1 eq.) was added, and the mixture was stirred for one minute before methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-chloro-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate[130] (0.485 g; 0.95 mmol; 2 eq.) was added as a solid. The reaction mixture was stirred overnight, then diluted with ethyl acetate (50 mL) and washed successively with 1M HCl, saturated sodium bicarbonate solution, and water, then dried over sodium sulfate, filtered and concentrated. Chromatography on a column of silica gel using hexanes/ethyl acetate/acetone (8:7:2) as eluent afforded **31** (0.309 g; 0.32 mmol; 67%): $[\alpha]_D$ 75 (c 0.71, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 8.14-8.11 (m, 2H, Ar), 7.56-7.50 (m, 3H, Ar), 7.47-7.43 (m, 2H, Ar), 7.36-7.30 (m, 3H, Ar), 5.62 (ddd, 1H, H-8'), 5.45 (s, 1H, C*H*Ph), 5.22 (dd, 1H, H-2, $J_{2,1}$ = 7.8 Hz, $J_{2,3}$ = 11.8 Hz), 5.20 (dd, 1H, H-7', J = 2.2 Hz, J = 10.0 Hz), 5.05 (d, 1H, NH, $J_{NH,H\text{-}5'}$ = 10.1 Hz), 4.95 (d, 1H, H-1, $J_{1,2}$), 4.80 (ddd, 1H, H-4'), 4.37-4.30 (m, 2H, H-9a', H-6a'), 4.09-4.03 (m, 2H, H-9b', H-6b'), 3.98 (ddd, 1H, H-a), 3.90-3.82 (m, 2H, H-5', H-3), 3.76 (bs, 4H, OMe, H-5), 3.73-

3.70 (m, 2H, H-4, H-6'), 3.59 (ddd, 1H H-a'), 2.56 (dd, 1H, H-3"$_{eq}$, $J_{3eq,3ax}$ = 12.7 Hz, $J_{3eq,4}$ = 4.6 Hz), 1.89 (dd, 1H, H-3"$_{ax}$, $J_{3ax,4}$ = 12.0 Hz), 2.20, 2.05, 1.96, 1.82, 1.67, (5 x C(O)CH_3), 0.82 (m, 2H, H-b, H-b'), -0.13 (s, 9H, Si(CH_3)$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 170.6, 170.21, 170.49, 170.10, 169.8, 165.8, 137.7, 132.7, 130.7, 130.1, 128.8, 128.2, 128.0, 126.3, 101.38, 101.33, 81.0, 76.2, 73.6, 69.4, 69.3, 69.2, 67.8, 67.5, 66.90, 66.86, 62.5, 52.9, 49.2, 45.7, 37.4, 23.1, 21.5, 20.8 (2C), 20.5, 18.1, -1.5; ESI HRMS calc'd for $C_{45}H_{59}NO_{18}SSiNa$: 984.3117. Found: 984.3118. Anal. calc'd for $C_{45}H_{59}NO_{18}SSi$: C 56.18, H 6.18, N 1.46. Found: C 55.75, H 6.39, N 1.38.

Allyl *S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-*galacto*-non-2-ulopyranosylonate)-(2→3)-2-*O*-benzoyl-(S)-4,6-*O*-benzylidene-3-thio-β-D-galactopyranoside (32)

The thiol **29** (0.613 g; 1.43 mmol) was dissolved in *N,N*-dimethylformamide (15 mL) and argon was bubbled through for one hour prior to cooling the solution to 0 °C. Sodium hydride (dispersion in mineral oil; 60 % by mass; 0.074 g; 1.85 mmol; 1.3 eq.) was added in portions and stirring was continued until the evolution of gas had ceased. A solution of methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-chloro-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate[130] (1.30 g; 2.55 mmol; 1.8 eq.) and 1,4,10-trioxa-7,13-diazacyclopentadecane (0.062 g; 0.29 mmol; 0.2 eq.) in *N,N*-dimethylformamide (5 mL) was added via canula and the mixture was stirred for 40 minutes, after which time the mixture was neutralized with acetic acid. The solution was diluted with ethyl acetate (150 mL) and washed with an equal volume of water, then dried over sodium sulfate, filtered and concentrated. Column chromatography on silica gel with a linear gradient of hexanes-ethyl acetate-acetone (4/3/1→4/3/2) afforded **32** (1.220 g; 1.35 mmol; 95%): $[\alpha]_D$ 50 (c 0.27, CHCl3); ^{1}H NMR (500 MHz, $CDCl_3$) δ

8.16-8.12 (m, 2H, Ar), 7.58-7.52 (m, 3H, Ar), 7.49-7.45 (m, 2H, Ar), 7.39-7.31 (m, 3H, Ar), 5.79 (dddd, 1H, H-b, J = 5.1 Hz, J = 5.9 Hz, J = 10.5 Hz, J = 17.2 Hz), 5.63 (ddd, 1H, H-8', J = 2.5 Hz, J = 5.6 Hz, $J_{8',7'}$ = 10.0 Hz), 5.48 (s, 1H, C*H*Ph), 5.30 (dd, 1H, H-2, $J_{2,1}$ = 7.7 Hz, $J_{2,3}$ = 11.8 Hz), 5.23 (dd, 1H, H-7', $J_{7',6'}$ = 2.2 Hz), 5.18 (dddd, 1H, H-c, J = 1.7 Hz, J = 1.7 Hz, J = 1.7 Hz, J = 17.2 Hz), 5.06-5.01 (m, 2H, H-1, H-d), 4.83 (ddd, 1H, H-4', $J_{4',3'eq}$ = 4.6 Hz, $J_{4',5'}$ = 10.4 Hz, $J_{4',3'ax}$ = 11.6 Hz), 4.38-4.31 (m, 3H, H-9a', H-6a, H-a), 4.15 (dddd, 1H, H-a', J = 1.4 Hz, J = 1.4 Hz, J = 6.0 Hz, J = 13.1 Hz), 4.12-4.05 (m, 2H, H-9b', H-6b), 3.93-3.85 (m, 2H, H-5', H-3), 3.80-3.73 (m, 3H, H-5, H-4, H-6'), 3.77 (s, 3H, CO_2CH_3), 2.58 (dd, 1H, H-3'$_{eq}$, $J_{3eq,3ax}$ = 12.9 Hz), 1.91 (1H, H-3'$_{ax}$), 2.21, 2.07, 1.97, 1.84, 1.70 (5s, 5 x 3H, 5 x C(O)CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 170.7, 170.18, 170.14, 170.12, 169.8, 165.9, 137.6, 134.1, 132.8, 130.6, 130.1, 128.9, 128.2, 128.1, 126.3, 116.9, 101.1, 100.9, 81.0, 76.3, 73.6, 69.6, 69.4, 69.2, 69.1, 67.9, 67.5, 66.9, 62.4, 53.0, 49.3, 45.6, 37.4, 23.2, 21.5, 20.8, 20.6; ESI HRMS calc'd for $C_{43}H_{51}NO_{18}SNa$: 924.2719. Found: 924.2720. Anal. calc'd for $C_{43}H_{51}NO_{18}S$: C 57.26, H 5.70, N 1.55. Found: C 56.87, H 5.56, N 1.40.

(2,4,6-Tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose (33)

To a stirring solution of (2,6-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-1,2,3,6-tetra-*O*-acetyl β-D-glucopyranose[279] (0.500 g; 0.79 mmol) in freshly distilled acetonitrile (10.0 mL) was added trimethylorthoacetate (0.32 mL; 2.5 mmol; 3.2 eq.), followed by *p*-toluenesulfonic acid monohydrate (0.015 g; 0.08mmol). After 30 min., 80% aqueous acetic acid (25.0 mL) was added and stirring was continued for 20 minutes. The reaction mixture was concentrated and the residue taken up in 95% ethanol (50 mL) and

concentrated again. The residue was crystallized from anhydrous ethanol to afford **33** (0.483 g; 0.76 mmol; 90%). $[\alpha]_D$ –1 (c 0.9, CHCl3); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.65 (d, 1H, H-1, $J_{1,2}$ = 8.2 Hz), 5.27 (d, 1H, H-4', $J_{4',3'}$= 3.3 Hz), 5.22 (dd, 1H, H-3, $J_{3,2}$ = $J_{3,4}$ 9.1 Hz), 5.03 (dd, 1H, H-2), 4.83 (dd, 1H, H-2', $J_{2',1'}$ = 7.9 Hz, $J_{2',3'}$ = 9.9 Hz), 4.40 (dd, 1H, H6a', $J_{6a',6b'}$ = 12.1 Hz, $J_{6b',5'}$ = 1.5 Hz), 4.39 (d, 1H, H-1'), 4.18 (dd, 1H, H-6b', $J_{6b',5'}$ = 4.9 Hz), 4.07 (m, 2H, H-6a, H-6b), 3.77 (m, 4H, H4, H5, H3', H5'), 2.15, 2.10, 2.09, 2.08, 2.07, 2.02, 2.01 (7s, 21H, 7xAc); ^{13}C NMR (125MHz, δ) ($CDCl_3$): 171.1, 170.6. 170.4, 170.3, 169.6, 169.5, 168.8, 100.58, 91.56, 75.67, 73.60, 73.0, 72.47, 71.68, 71.02, 70.45, 69.15, 61.86, 61.44, 20.79, 20.77 (2C), 20.71, 20.69, 20.64, 20.55; ESI HRMS calc'd for $C_{26}H_{36}O_{18}NNa$: 659.1799. Found: 659.1799. Anal. calc'd for $C_{26}H_{36}O_{18}N$: C 49.06, H 5.70. Found: C 48.99, H 5.56.

(2,4,6-Tri-*O*-acetyl-3-*O*-trifluoromethanesulfonyl-β-D-gulopyranosyl)-(1→4)-1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose (34) and **(2,4,6-Tri-*O*-acetyl-β-D-gulopyranosyl)-(1→4)-1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose (35)**

Alcohol **33** (2.025 g; 3.2 mmol) was dissolved in freshly distilled dichloromethane (30.0 mL) and pyridine (1.0 mL; 12.0 mmol; 3.75 eq.) with stirring under Ar. The solution was cooled to –35 °C and trifluoromethanesulfonic anhydride (1.07 mL; 6.4 mmol; 2.0 eq.) was added dropwise over a period of one minute. After 30 minutes the reaction mixture was diluted with dichloromethane (120 mL), and then washed successively with 1M HCl, saturated $NaHCO_3$, and water. The organic layers were dried over sodium sulfate, filtered and concentrated (bath temp. < 20 °C). A portion of the triflate was passed through a column of silica gel with ethyl acetate/toluene (2:1) as eluent to afford a pure sample of **34**. $[\alpha]_D$ –2 (c 0.91, CHCl3); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.65 (d, 1H, H-1, $J_{1,2}$ = 8.2 Hz), 5.53 (bd, 1H, H-4', $J_{4',3'}$ = 3.6 Hz), 5.21 (dd, 1H, H-3, $J_{3,2}$ = $J_{3,4}$ = 9.2

Hz), 5.24-5.18 (m, 2H, H-3, H-2'), 5.01 (dd, 1H, H-2), 4.85 (dd, 1H, H-3', $J_{3',2'}$ = 10.2 Hz), 4.49 (d, 1H, H-1', $J_{1',2'}$ = 7.9 Hz), 4.41 (dd, 1H, H-6a, $J_{6a,5}$ = 2.0 Hz, $J_{6a,6b}$ = 12.1 Hz), 4.13-4.05 (m, 3H, H-6a', H-6b', H-6b), 3.87-3.79 (m, 2H, H-5', H-4), 3.74 (ddd, 1H, H-5, $J_{5,6b}$ = 4.9 Hz, $J_{5,4}$ = 9.8 Hz), 2.16, 2.09, 2.08, 2.07, 2.06, 2.02, 2.00 (7s, 21H, 7 x C(O)CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.31, 170.26, 169.49, 169.47, 169.3, 168.8, 168.5, 118.2 (q, CF_3, $J_{C,F}$ = 320 Hz), 100.3, 91.4, 82.6, 75.8, 73.4, 72.5, 70.44, 70.42, 68.5, 66.8, 61.7, 60.6, 20.75, 20.72, 20.67, 20.54, 20.51, 20.30, 20.29; ESI HRMS calc'd for $C_{52}H_{55}O_{15}Na$: 791.1292, found: 791.1302. The crude triflate was dissolved in dry, degassed *N,N*-dimethylformamide (30 mL) and tetrabutylammonium nitrite (3.670 g; 12.7 mmol) was added. After stirring overnight at 30 °C, the solution was concentrated, and the residue chromatographed on silica gel using ethyl acetate-hexanes (5:4) as eluent to yield **35** (1.815 g; 2.8 mmol; 90%): $[\alpha]_D$ –22 (c 1.3, CHCl3); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.66 (d, 1H, H-1, $J_{1,2}$ = 8.3 Hz), 5.21 (dd, 1H, H-3, $J_{3,2}$ = $J_{3,4}$ 9.2 Hz), 5.04 (dd, 1H, H-2), 4.88 (dd, 1H, H-4', $J_{4',3'}$ = 3.8 Hz, $J_{4',5'}$ = 1.4 Hz), 4.78 (d, 1H, H-1', $J_{1',2'}$ 8.4 Hz), 4.70 (dd, 1H, H2', $J_{2',3'}$ = 3 Hz), 4.38 (dd, 1H, H-6a, $J_{6a,6b}$ = 12.2 Hz, $J_{6a,5}$ = 2.0 Hz), 4.28 (dd, 1H, H-6b, $J_{6b,5}$ 2.9 Hz), 4.23 (m, 1H, H-5), 4.16 (m, 1H, H3'), 4.08 (m, 2H, H6a', H6b'), 3.82 (dd, H4, $J_{4,3} \approx J_{4,5} \approx$ 9.5 Hz), 2.10, 2.09, 2.08, 2.07, 2.05, 2.02. 2.00 (7s, 21H, 7xAc); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5, 170.4, 169.9, 169.7, 169.4, 169.1, 168.8, 98.6, 91.7, 76.0, 73.7, 72.6, 71.0, 70.5, 69.8, 69.7, 67.2, 62.1, 61.6. 20.9-20.6 (7C); ESI HRMS calc'd for $C_{26}H_{36}O_{18}NNa$: 659.1799. Found: 659.1797. Anal. calc'd for $C_{26}H_{36}O_{18}N$: C 49.06, H 5.70. Found: C 49.16, H 5.80.

(3,4,6-Tri-*O*-acetyl-β-D-gulopyranosyl)-(1→4)-1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose (36)

^{1}H NMR (600 MHz, $CDCl_3$) δ 5.69 (d, 1H, H-1, $J_{1,2}$ = 8.4 Hz), 5.28 (m, 2H, H-3, H-3'), 4.90 (bd, 1H, H-4', $J_{4',3'}$ = 3.5 Hz), 4.54 (d, 1H, H-1', $J_{1',2'}$ = 8.1 Hz), 4.44 (dd, 1H, H-6a, $J_{6a,5}$ = 4.2 Hz, $J_{6a,6b}$ = 12.4 Hz), 4.17 (m, 1H, H-6b), 4.09-4.03 (m, 3H, H-6a', H-6b', H-5'), 3.84-3.79 (dd, 2H, H-4, H-5), 3.69 (ddd, 1H, H-2', $J_{2',1'}$ = 8.0 Hz, $J_{2',3'}$ = 3.9 Hz, $J_{2',OH\text{-}2'}$ = 4.8 Hz), 2.13, 2.10, 2.09, 2.08, 2.06, 2.03, 2.01 (7s, 21H, 7xC(O)CH_3); ESI HRMS calc'd for $C_{26}H_{36}O_{18}NNa$: 659.1799. Found: 659.1800. Anal. calc'd for $C_{26}H_{36}O_{18}N$: C 49.06, H 5.70. Found: C 48.60, H 5.66.

(2,4,6-Tri-*O*-acetyl-3-*S*-acetyl-3-thio-β-D-galactopyranosyl)-(1→4)-1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose (37)

Alcohol **35** (0.351 g; 0.55 mmol) was dissolved in freshly distilled dichloromethane (25.0 mL) and pyridine (0.535 mL; 6.6 mmol; 12.0 eq.) with stirring under an argon atmosphere. The solution was cooled to –10 °C, and trifluoromethanesulfonic anhydride (0.745 mL; 4.41 mmol; 8.0 eq.) was added dropwise over a period of several minutes. After 1 hour, the reaction mixture was diluted with dichloromethane (30 mL), washed successively with ice cold 1M HCl, saturated $NaHCO_3$ solution, and water, and the organic layers dried over sodium sulfate, then filtered. The filtrate was concentrated to ~2 mL (bath temp. < 20 °C) and the residue combined with dry toluene. This solution was then transferred to a flask containing crude tetrabutylammonium thioacetate (30 eq.). After stirring for four hours, the mixture was concentrated. Column chromatography on silica gel using methanol-dichloromethane (1:200) as eluent afforded thioester **37** (0.203 g; 0.29 mmol; 53%): $[\alpha]_D$ 13 (c 1.07, CHCl3); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.64 (d, 1H, H1, $J_{1,2}$ = 8.3 Hz), 5.23 (dd, 1H, H4', $J_{4',3'}$ = 3.1 Hz, $J_{4',5'}$ = 1.1 Hz), 5.21 (dd,

1H, H3, $J_{3,2} \approx J_{3,4} \approx 9.3$ Hz), 5.02 (dd, 1H, H2), 4.92 (dd, 1H, H2', $J_{2',1'} = 7.8$ Hz, $J_{2',3'} =$ 11.9 Hz), 4.51 (d, 1H, H1'), 4.44 (dd, 1H, H6a, $J_{6a,6b} = 12.1$ Hz, $J_{6a,5} = 2.0$ Hz), 4.10 (dd, 1H, H6b, $J_{6b,5}$ 5.1 Hz), 4.01 (m, 2H, H6a', H6b'), 3.95 (m, 1H, H5'), 3.83 (m, 2H, H3', H4), 3.73 (m, 1H, H5), 2.28, 2.12, 2.11, 2.08, 2.06 (5s, 15H, 5xAc), 2.02 (s, 6H, 2xAc), 2.01 (s, 3H, Ac); ^{13}C NMR (125 MHz, $CDCl_3$) δ 193.6, 170.2, 170.1, 169.7, 169.4, 169.3, 169.1, 168.7, 101.9, 91.6, 75.4, 73.6, 73.3, 72.6, 70.6, 68.2, 67.8, 61.8, 61.5, 47.3, 30.6, 20.92, 20.88, 20.81, 20.74, 20.66, 20.65, 20.64; ESI HRMS calc'd for $C_{28}H_{38}O_{18}S$: 717.1675. Found: 717.1677. Anal. calc'd for $C_{28}H_{38}O_{18}S$: C 48.41, H 5.51. Found: C 48.66, H 5.69.

4-*S*-Acetyl-1,2,3,6-tetra-*O*-benzoyl-4-thio-α/β-D-galactopyranose (39)

1,2,3,6-tetra-*O*-benzoyl-α/β-D-glucopyranose[155] (0.126 g; 0.21 mmol) was dissolved in dichloromethane (5.0 mL) and pyridine (0.102 mL; 1.27 mmol; 6.0 eq.) with stirring at –20 °C. Trifluoromethanesulfonic anhydride (0.089 mL; 0.53 mmol; 2.5 eq.) was added to the solution, which was then warmed to 0 °C. After several hours, the reaction mixture was diluted with dichloromethane (15 mL), washed with water (15 mL), dried over sodium sulfate, filtered, and concentrated. The residue was combined with potassium thioacetate (0.121 g; 1.04 mmol; 5 eq.) in *N,N*-dimethylformamide (4 mL) and heated at 40 °C for 5 hours. The mixture was diluted with ethyl acetate (25 mL), washed with water (2 x 15 mL), dried over sodium sulfate, filtered, and concentrated. Chromatography of the residue on silica gel using hexanes/ethyl acetate (5:2) as eluent afforded **39** (0.115 g; 0.18 mmol; 83 %): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.12-7.27 (Ar H), α-anomer: 6.78 (d, 1H, H-1, $J_{1,2} = 3.9$ Hz), 6.14 (dd, 1H, H-3, $J_{3,4} = 4.5$ Hz, $J_{3,2} =$ 10.6 Hz), 5.73 (dd, 1H, H-2, $J_{2,1} = 3.8$ Hz), 4.97 (ddd, 1H, H-5, $J = 6.0$ Hz, $J = 7.0$ Hz, J

= 1.6 Hz), 4.83 (dd, 1H, H-4), 4.56 (dd, 1H, H-6a, $J_{6a,5}$ = 7.0 Hz, $J_{6a,6b}$ = 11.6 Hz), 4.41 (dd, 1H, H-6b, $J_{6b,5}$ = 6.0 Hz), 2.32 (s, 3H, SC(O)CH_3), β-anomer: 6.20 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 5.81-5.78 (m, 2H, H-2, H-3), 4.77 (bd, 1H, H-4), 4.65-4.60 (m, 2H, H-6a, H-5), 4.47 (dd, 1H, H-6b, J = 14.6 Hz, 9 Hz); ^{13}C NMR (125 MHz, acetone-d_6, from HMQC) δ α-anomer: C-1 91.4, SC(O)CH_3 30.7, β-anomer: C-1 94.0, SC(O)CH_3 30.6 ; ESI HRMS calc'd for $C_{36}H_{30}O_{10}SNa$: 677.1452. Found: 677.1456. Anal. calc'd for $C_{36}H_{30}O_{10}S$: C 66.05, H 4.62. Found: C 66.22, H 4.75.

4-*S*-Acetyl-1,2,3,6-tetra-*O*-benzoyl-4-thio-α/β-D-glucopyranose (40)

1,2,3,6-Tetra-*O*-benzoyl-α/β-D-galactopyranose[154] (0.528 g; 0.89 mmol) was dissolved in dichloromethane (5.0 mL) and pyridine (0.286 mL; 3.54 mmol; 4.0 eq.), then cooled to 0 °C under argon. Trifluoromethanesulfonic anhydride (0.300 mL; 1.77 mmol; 2.0 eq.) was added dropwise to the stirring solution. After 1.5 hours, the reaction mixture was diluted with dichloromethane (25 mL) and washed with water (2 x 15 mL), then dried over sodium sulfate, filtered, and concentrated. The residue was combined with potassium thioacetate (0.500 g; 4.40 mmol; 5.0 eq.) and *N,N*-dimethylformamide was added. The mixture was stirred at 40 °C for 4 hours, then diluted with ethyl acetate (25 mL), washed with water (2x30 mL), dried over sodium sulfate, filtered, and concentrated. The residue was purified on silica gel using hexanes/ethyl acetate (7:2) as eluent to afford **40** as a mixture of anomers (0.467 g; 0.71 mmol; 80%): ^{1}H NMR (600 MHz, $CDCl_3$) δ α-anomer: 8.17-8.14 (m, 2H, Ar), 8.12-8.09 (m, 2H, Ar), 7.97-7.94 (m, 2H, Ar), 7.88-7.85 (m, 2H, Ar), 7.67-7.63 (m, 1H, Ar), 7.62-7.58 (m, 1H, Ar), 7.55-7.43 (m, 6H, Ar), 7.41-7.36 (m, 2H, Ar), 7.32-7.27 (m, 2H, Ar), 6.85 (d, 1H, H-1, $J_{1,2}$ = 3.6 Hz), 6.19 (dd, 1H, H-3, $J_{3,2}$ = 10.0 Hz, $J_{3,4}$ = 11.1 Hz), 5.62 (dd, 1H, H-2), 4.66-4.57 (m,

3H, H-5, H-6a, H-6b), 4.24 (dd, 1H, H-4, $J_{4,5}$ = 11.2 Hz), 2.24 (s, 3H, $SC(O)CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.3, 166.2, 165.8, 165.4, 164.4, 133.8, 133.43, 133.39, 133.2, 130.0, 129.8 (2C), 129.0, 128.9, 128.7, 128.6, 128.45, 128.44 (2C), 128.36, 90.3, 71.6, 71.1, 69.0, 63.4, 44.0, 30.7; ESI HRMS calc'd for $C_{36}H_{30}O_{10}SNa$: 677.1452. Found: 677.1454. Anal. calc'd for $C_{36}H_{30}O_{10}S$: C 66.05, H 4.62. Found: C 66.29, H 4.68.

2-(Trimethylsilyl)ethyl (2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (41)

To a solution of 2-(trimethylsilyl)ethyl (3-*O*-(4-methoxybenzyl)-β-D-galactopyranosyl)-(1-4)-β-D-glucopyranoside[156] (0.186 g; 0.33 mmol) in dry *N,N*-dimethylformamide (6.0 mL) was added benzaldehyde dimethyl acetal (95 μl; 0.63 mmol; 1.9 eq.). *p*-Toluenesulfonic acid monohydrate (10 mg) was added, and the mixture was stirred for 13 hours at room temperature, then neutralized with triethylamine and concentrated. The crude residue was taken up in pyridine (5 mL) and benzoyl chloride (305 μL; 2.64 mmol; 8 eq.), and stirred overnight. The mixture was concentrated, and the residue dissolved in dichloromethane (45 mL), washed with 1M HCl, followed by saturated $NaHCO_3$ solution and water, then dried over sodium sulfate. Following evaporation to dryness, the residue was chromatographed in toluene/ethyl acetate (10:1) to afford **41** (0.215 g, 70%): $[\alpha]_D$ 75 (c 0.83, CHCl3); ^1H NMR (600 MHz, $CDCl_3$) δ 7.96-7.83 (8H, Ar), 7.56-7.21 (17H, Ar), 7.01 (2H, Ar), 6.63 (2H, Ar), 5.75 (t, 1H, H-3, $J_{2,1} = J_{2,3}$ = 9.1 Hz), 5.49 (dd, 1H, H-2', $J_{2',1'}$ = 8.1 Hz, $J_{2',3'}$ = 10.1 Hz), 5.27 (dd, 1H, H-2, $J_{2,1}$ = 7.8 Hz, $J_{2,3}$ = 9.3 Hz), 5.25 (s, 1H, C*H*Ph), 4.65 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 4.63 (d, 1H, H-1', $J_{1',2'}$ = 8.0 Hz), 4.57 (dd, 1H, H-6a, $J_{6a,6b}$ = 11.8 Hz, $J_{6a,5}$ = 2.0 Hz), 4.44 (d, 1H, *J* = 12.5 Hz, CH_2PhOCH_3), 4.40-

4.33 (m, 2H, H-6b, CH_2PhOMe), 4.09 (t, 1H, H-4, $J_{4,3} = J_{4,5} = 9.1$ Hz), 3.92 (d, 1H, H-4', $J_{4',3'} = 3.1$ Hz), 3.86-3.77 (m, 2H, H-a, H-5), 3.71 (s, 3H, CH_2PhOCH_3), 3.67 (d, 1H, H-6a', $J_{6a'-6b'} = 11.58$ Hz), 3.65-3.44 (m, 3H, H-6b', H3', H-a'), 2.75 (s, 1H, H5'), 0.76 (m, 2H, H-b, H-b'), -0.19 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.5, 165.2, 164.81, 164.76, 159.0, 137.5, 132.84, 132.78, 132.74, 132.73, 129.78, 129.74, 129.67, 129.66, 129.65, 129.56, 129.52, 129.0, 129.4, 129.0, 128.7, 128.17, 128.14, 128.12, 128.11, 127.9, 126.5, 113.6, 101.63, 100.90, 100.02, ~77.0, 76.5, 74.2, 72.8, 72.6, 72.4, 71.2, 70.4, 68.2, 67.4, 66.7, 62.8, 55.2, 17.90, -1.48; ESI HRMS calc'd for $C_{60}H_{63}O_{16}SiNa$: 1089.3705. Found: 1089.3697. Anal. calc'd for $C_{60}H_{63}O_{16}Si$: C 67.46, H 5.94. Found: C 67.32 H 5.84.

2-(Trimethylsilyl)ethyl-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (42)

Compound **41** (3.980 g; 3.7 mmol) was dissolved in 95% aqueous acetonitrile (125 mL), and ceric ammonium nitrate (12.267 g; 22.4 mmol; 6.1 eq.) was added. The solution was stirred at room temperature for 40 minutes and was then diluted with dichloromethane (500 mL), washed with water (2x250 mL), dried over sodium sulfate, and concentrated. Crystallization of the solid residue from acetonitrile/water afforded compound **42** (3.032 g; 3.20 mmol; 86%). Column chromatography of the mother liquor in ethyl acetate/chloroform (1:49) yielded additional product (0.254 g; 0.26 mmol; 7%): $[\alpha]_D$ 29 (c 0.73, CHCl3); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02-7.88 (8H, Ar), 7.60-7.24 (17H, Ar), 5.75 (t, 1H, H-3, $J_{3,2} = J_{3,4} = 9.1$ Hz), 5.32 (dd, 1H, H-2, $J_{2,1} = 7.8$ Hz, $J_{2,3} = 9.6$ Hz), 5.30 (s, 1H, C*H*Ph), 5.27 (dd, 1H, H-2', $J_{2',1'} = 8.0$ Hz, $J_{2',3'} = 10.0$ Hz), 4.67 (d, 1H, H-1, $J_{1,2} = 7.8$ Hz), 4.63 (d, 1H, H-1', $J_{1',2'}$, = 7.9 Hz), 4.57 (dd, 1H, $J_{6a,6b} = 11.8$ Hz, $J_{6a,5} = 2.1$

Hz, H-6a), 4.44 (dd, 1H, $J_{6b,6a}$ = 11.8 Hz, $J_{6b,5}$ = 4.9 Hz, H-6b), 4.12 (t, 1H, H-4, $J_{4,3}$ = $J_{4,5}$ = 9.5 Hz), 3.97 (d, 1H, H-4', $J_{4',3'}$ = 3.2 Hz), 3.83 (m, 2H, H-5, H-a), 3.70 (dd, 1H, $J_{3',2'}$ = 10.6 Hz, H-3', $J_{3',4'}$ = 3.2 Hz), 3.65 (dd, 1H, $J_{6a',6b'}$ = 12.4 Hz, $J_{6a',5'}$ = 1.0 Hz, H-6a'), 3.57 (dd, 1H, $J_{6b',6a'}$ = 12.4, $J_{6b',5'}$ = 1.8 Hz, H-6b'), 3.50 (ddd, 1H, H-a'), 2.93 (d, 1H, H-5', $J_{5',6a'}$ = 1.0 Hz), 2.38 (d, 1H, $J_{3'OH,3'}$ = 11.6 Hz, O*H*3'), 0.81 (m, 2H, H-b, H-b'), -0.17 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.7, 165.6, 165.2, 164.9, 137.2, 133.1, 133.0, 132.9, 132.82, 129.76, 129.73, 129.70, 129.69, 129.65, 129.60, 129.5, 129.2, 129.1, 128.28, 128.25, 128.157, 128.151, 128.0, 126.5, 101.23, 101.19, 100.2, 76.95, 75.7, 73.8, 73.1, 72.3, 71.5, 69.2, 68.2, 67.4, 65.8, 62.8, 17.9, -1.5. ESI HRMS calc'd for $C_{52}H_{55}O_{15}SiNa$: 969.3130, Found: 969.3131. Anal. calc'd for $C_{52}H_{55}O_{15}Si$: C 65.95, H 5.75. Found: C 65.95, H 5.75.

2-(Trimethylsilyl)ethyl-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-gulopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (43)

Crystalline **42** (0.918 g; 0.97 mmol) was dissolved in freshly distilled dichloromethane (20 mL) and pyridine (0.470 mL; 5.81 mmol; 6.0 eq.) with stirring under an argon atmosphere. The solution was cooled to –40 °C, and trifluoromethanesulfonic anhydride (0.650 mL; 3.88 mmol; 4.0 eq.) was added dropwise over approximately one minute. After 70 minutes at constant temperature, the reaction mixture was diluted with dichloromethane (40 mL) and washed successively with 1M HCl, saturated $NaHCO_3$ solution, and water. The organic layer was then dried over sodium sulfate, filtered, and concentrated. The crude residue was taken up in *N,N*-dimethylformamide (15 mL), and tetrabutylammonium nitrite (2.21 g; 7.76 mmol; 8.0 eq.) was added. The mixture was stirred overnight at room temperature and then evaporated to dryness. The residue was

taken up in dichloromethane (20 mL), washed with water, dried over sodium sulfate, and then concentrated. Chromatography in methanol/dichloromethane (1:99) gave the title compound **43** (0.837 g; 0.88 mmol; 91%): $[\alpha]_D$ 5 (c 0.84, CHCl3); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14-7.89 (8H, Ar), 7.60-7.22 (17H, Ar), 5.74 (t, 1H, H-3, $J_{3,2} = J_{3,4} = 9.5$ Hz), 5.33 (dd, 1H, H-2, $J_{2,1} = 7.8$ Hz, $J_{2,3} = 9.6$ Hz), 5.30-5.27 (m, 2H, H-2', C*H*Ph), 5.05 (d, 1H, H-1', $J_{1',2'} = 8.4$ Hz), 4.67 (d, 1H, H-1, $J_{1,2} = 7.9$ Hz), 4.59 (dd, 1H, $J_{6a,6b} = 11.9$ Hz, $J_{6a,5} = 4.6$ Hz, H-6a), 4.54 (dd, 1H, $J_{6b,6a} = 11.9$ Hz, $J_{6b,5} = 2.3$ Hz, H-6b), 4.21 (t, 1H, H-3', $J_{3',2'} = J_{3',4'} = 3.1$ Hz), 4.13 (t, 1H, H-4, $J_{4,3} = J_{4,5} = 9.5$ Hz), 3.88 (ddd, 1H, H-a), 3.83-3.78 (m, 2H, H-4', H-5), 3.62 (dd, 1H, $J_{6a',6b'} = 11.8$ Hz, $J_{6a',5'} = 1.1$ Hz), 3.57-3.47 (m, 2H, H-6b, H-a'), 3.29 (d, 1H, H-5', $J_{5',6a'} = 1.1$ Hz), 0.80 (ddd, 2H, H-b, H-b'), -0.15 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.9, 165.20, 165.18, 165.12, 137.6, 133.3, 133.0, 132.9, 132.7, 129.75, 129.74, 129.71, 129.65 (2C), 129.55, 129.1, 128.8, 128.5, 128.3, 128.13, 128.08, 127.92, 127.90, 126.4, 101.0, 100.26, 98.50, 76.8, 75.7, 73.8, 73.1, 72.3, 71.5, 69.2, 68.2, 67.4, 65.8, 62.8, 18.0, -1.4; ESI HRMS calc'd for $C_{52}H_{55}O_{15}SiNa$: 969.3130. Found: 969.3132. Anal. calc'd for $C_{52}H_{55}O_{15}Si$: C 65.95, H 5.75. Found: C 66.05, H 5.74.

2-Trimethylsilyl(ethyl) (3-*S*-acetyl-2-*O*-benzoyl-4,6-*O*-benzylidene-3-thio-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (44) and **2-Trimethylsilyl(ethyl) (2-*O*-benzoyl-4,6-*O*-benzylidene-2,3-dideoxy-β-D-*threo*-hex-2-enopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (44)**

2-(Trimethylsilyl)ethyl-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-gulopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside **43** (0.615 g; 0.65 mmol) was dissolved in dichloromethane (10.0 mL) containing pyridine (1.05 mL; 13 mmol; 20 eq.). The solution was cooled to –65 °C and trifluoromethanesulfonic anhydride (0.41 mL, 3.81

mmol, 12.0 eq.) was added dropwise over a period of roughly one minute. The reaction mixture was allowed to warm to room temperature over 30 minutes. After stirring for a total of 1 hour, dichloromethane (50 mL) was added, and solution was washed with water (75 mL), dried over sodium sulfate, filtered, and concentrated (<25 °C bath temperature). The residue was taken up in *N,N*-dimethylformamide (10.0 mL), and potassium thioacetate (0.445 g; 3.90 mmol; 6.0 eq.) was added. The suspension was stirred for one hour at 70 °C, then concentrated. The residue was dissolved in ethyl acetate (50 mL), washed with water (3x50 mL), dried over sodium sulfate, filtered, and concentrated. The residue was chromatographed on silica gel using a mixture of hexanes/ethyl acetate (7:2 → 11:4) as eluent, affording **44** (0.516 g; 0.51 mmol; 79%): $[\alpha]_D$ 118 (c 0.8, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.0-7.8 (8H, Ar), 7.7-7.2 (17H, Ar), 5.79 (dd, 1H, H-3, $J_{3,2} = J_{3,4} = 9.0$ Hz), 5.36 (dd, 1H, H-2', $J_{2',1'} = 7.9$ Hz, $J_{2',3'} = 11.5$ Hz), 5.29 (s, 1H, C*H*Ph), 5.26 (dd, 1H, H-2, $J_{2,1} = 7.6$ Hz, $J_{2,3} = 9.3$ Hz), 4.81 (d, 1H, H-1), 4.65 (d, 1H, H-1'), 4.59 (dd, 1H, H-6a, $J_{6a,5} = 2.2$ Hz, $J_{6a,6b} = 11.7$ Hz), 4.26 (dd, 1H, H-6b, $J_{6b,5} = 4.6$ Hz), 4.16 (dd, 1H, H-4, $J_{4,3} = J_{4,5} = 9.0$ Hz), 4.04 (dd, 1H, H-3') 3.81 (m, 3H, H-4', H-5, H-a), 3.77 (dd, 1H, H-6a', $J_{6a',5'} = 1.3$ Hz, $J_{6a',6b'} = 12.5$ Hz), 3.48 (m, 2H, H-6b', H-a'), 2.96 (m, 1H, H-5'), 2.08 (s, 3H, C(O)CH_3), 0.76 (m, 2H, H-b, H-b'), -0.19 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.8, 165.5, 165.3, 165.1, 164.8, 137.3, 133.1, 133.0, 132.9, 132.8, 129.81, 129.76, 129.70, 129.66, 129.62, 129.61, 129.58, 128.84, 128.83, 128.4, 128.3, 128.2, 128.1, 128.0, 126.3, 102.6, 100.9, 100.0, 76.8, 75.3, 74.4, 72.7, 72.5, 69.5, 68.4, 68.0, 67.3, 62.4, 46.8, 30.4, 17.8, -1.6; ESI HRMS calc'd for $C_{54}H_{56}O_{15}SSi$: 1027.3007. Found: 1027.3002. Anal. calc'd for $C_{54}H_{56}O_{15}SSi$: C 64.52, H 5.62. Found: C 64.34, H 5.71. A side product (**45**) was also

observed on several occasions: $[\alpha]_D$ 4 (c 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.00-7.87 (8H, Ar), 7.59-7.32 (15H, Ar), 7.25-7.22 (m, 2H, Ar), 5.85 (dd, 1H, H-3, $J_{3,2}$ = $J_{3,4}$ = 9.2 Hz), 5.84 (d, 1H, H-3', $J_{3',4'} \sim J_{3',2'} \sim$ 6.3 Hz), 5.73 (s, 1H, H-1'), 5.47 (s, 1H, C*H*Ph), 5.28 (dd, 1H, H-2, $J_{2,1}$ = 7.9 Hz, $J_{2,3}$ = 9.3 Hz), 4.90 (dd, 1H, H-6a, $J_{6a,5}$ = 2.4 Hz, $J_{6a,6b}$ = 12.0 Hz), 4.74 (d, 1H, H-1), 4.57 (dd, 1H, H-4, $J_{4,5}$ = 9.2 Hz), 4.38 (dd, 1H, H-6b, $J_{6b,5}$ = 6.2 Hz), 4.08 (d, 1H, H-6a', $J_{6a',6b'}$ = 12.9 Hz), 3.96-3.87 (m, 2H, H-5, H-a), 3.84 (dd, 1H, H-6b', $J_{6b',5'}$ = 2.4 Hz), 3.55 (ddd, 1H, H-a', *J* = 9.8 Hz, *J* = 9.9 Hz, *J* = 6.4 Hz), 3.35 (bs, 1H, H-5'), 0.88 (ddd, 1H, H-b, *J* = 6.4 Hz, *J* = 10.7 Hz, *J* = 13.9 Hz), 0.81 (ddd, 1H, H-b', *J* = 5.7 Hz, *J* = 10.5 Hz, *J* = 13.9 Hz), -0.15 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.8, 165.22, 165.17, 164.4, 149.6, 137.7, 133.8, 132.9, 132.8(2C), 130.14, 130.07, 129.82, 129.78, 129.72, 129.68, 129.61, 129.1, 128.5, 128.27, 128.23, 128.17, 128.15, 128.12, 126.5, 114.4, 101.1, 100.1, 95.7, 74.1, 73.7, 73.3, 72.9, 69.1, 68.9, 67.2, 66.7, 63.6, 17.9, -2.0; ESI HRMS calc'd for $C_{52}H_{52}O_{14}SiNa$: 951.3019. Found: 951.3018.

2-(Trimethylsilyl)ethyl *S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonate)-(2→3)-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-thio-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (47)

The thioester **44** (0.313 g; 0.31 mmol) was dissolved in dry, degassed *N,N*-dimethylformamide (5.0 mL) and argon was bubbled through the solution. Hydrazine acetate (0.057 g; 0.62 mmol; 2.0 eq.) was added and the mixture was stirred for 1.5 hours, then diluted with ethyl acetate (100 mL), washed with water, dried over sodium sulfate, and concentrated. The residue was dissolved in degassed dichloromethane and passed through a short plug of silica gel (4cm), using degassed hexanes/ethyl acetate

(2:1) as the mobile phase. ^{1}H NMR data were recorded for the intermediate thiol **46**: (600 MHz, $CDCl_3$) δ 8.01-7.86 (m, 8H, Ar), 7.57-7.21 (m, 17H, Ar), 5.75 (dd, 1H, H-3, $J_{3,2}$ = 9.2 Hz, $J_{3,4}$ = 9.2 Hz), 5.31 (s, 1H, C*H*Ph), 5.29 (dd, 1H, H-1, $J_{2,1}$ = 7.9 Hz), 5.22 (dd, 1H, H-2', $J_{2',1'}$ = 7.8 Hz, $J_{2',3'}$ = 11.0 Hz), 4.66 (d, 1H, H-1), 4.65 (d, 1H, H-1'), 4.54 (dd, 1H, H-6a, $J_{6a,5}$ = 2.0 Hz, $J_{6a,6b}$ = 11.8 Hz), 4.37 (dd, 1H, H-6b, $J_{6b,5}$ = 4.8 Hz), 4.13 (dd, 1H, H-4, $J_{4,5}$ = 9.3 Hz), 3.87-3.81 (m, 2H, H-4', H-a), 3.79 (ddd, 1H, H-5), 3.71 (dd, 1H, H-6a', $J_{6a',5'}$ = 0.9 Hz), $J_{6a',6b'}$ = 12.2 Hz), 3.55 (dd, 1H, H-6b', $J_{6b',5'}$ = 1.6 Hz), 3.48 (ddd, 1H, H-a', *J* = 6.4 Hz, *J* = 10.4 Hz, *J* = 10.2 Hz), 2.95-2.90 (m, 2H, H-3', H-5'), 2.08 (d, 1H, SH, $J_{SH,H-3}$ = 11.2 Hz), 0.81 (ddd, 1H, H-b, *J* = 6.6 Hz, *J* = 10.7 Hz, *J* = 14.1 Hz), 0.74 (ddd, 1H, H-b, *J* = 5.7 Hz, *J* = 10.4 Hz, *J* = 14.1 Hz), -0.18 (s, 9H, $Si(CH_3)_3$). The residue was combined with 1,4,10-trioxa-7,13-diazacyclopentadecane (0.010 g; 0.05 mmol; 0.15 eq.) and dissolved in dry, degassed *N,N*-dimethylformamide (5.0 mL). Sodium hydride (12.5 mg; 60% dispersion in oil; 1.5 eq.) was added, followed after 10 minutes by solid 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-chloro-3,5-dideoxy-*D-glycero*-β-*D-galacto*-non-2-ulopyranosylonate[130] (0.210 g; 0.41 mmol; 1.5 eq.). After stirring overnight, the reaction mixture was diluted with ethyl acetate (50 mL), washed with saturated sodium bicarbonate solution, then water, and the organic layers dried over sodium sulfate and concentrated. The residue was chromatographed on silica gel using a gradient of 1% to 2% methanol in dichloromethane as eluent, affording **47** (0.292 g; 0.20 mmol; 65%): $[\alpha]_D$ 71 (c 0.83, CHCl3); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.14-8.10 (m, 2H, Ar), 7.96-7.92 (m, 4H, Ar), 7.89-7.86 (m, 2H, Ar), 7.43-7.17 (m, 15H, Ar), 5.72 (dd, 1H, H-3, $J_{3,2}$ = $J_{3,4}$ = 9.3 Hz), 5.65 (ddd, 1H, H-8", $J_{8",7"}$ = 10.1 Hz, $J_{8",9a"}$ = 2.5 Hz, $J_{8",9b"}$ = 4.8 Hz), 5.27 (dd, 1H, H-2, $J_{2,1}$ = 7.8 Hz, $J_{2,3}$ = 9.6 Hz), 5.23 (dd, 1H, H-7, $J_{7,6}$ = 2.4 Hz),

5.21-5.14 (m, 3H, C*H*Ph, H-1', H-2'), 4.98 (d, 1H, N*H*, $J_{NH,5''}$ = 9.9 Hz), 4.77 (ddd, 1H, H-4'', $J_{4'',5''}$ = 10.4 Hz, $J_{4'',3'e}$= 4.6, $J_{4'',3a''}$ = 11.7 Hz), 4.62 (d, 1H, H-1), 4.68 (dd, 1H, H-6a, $J_{6a,5}$ = 2.3 Hz, $J_{6a,6b}$ = 11.8 Hz), 4.40 (dd, 1H, H-9a'', $J_{9a'',9b''}$ = 12.8 Hz), 4.31-4.25 (m, 2H, H-4, H-6b), 4.19 (dd, 1H, H-9b''), 3.86-3.73 (m, 5H, H-a, H-5, H-3', H-4', H-5''), 3.69-3.65 (m, 4H, OC*H*$_3$, H-6''), 3.57 (dd, 1H, H-6a', $J_{6a',6b'}$ = 12.2 Hz $J_{6a',5'}$ = 1.85 Hz), 3.47-3.41 (m, 2H, H-a', H-6b'), 3.30 (m, 1H, H-5'), 2.49 (dd, 1H, H-3e'', $J_{3e'',3a''}$ = 12.7 Hz), 2.13, 1.98, 1.92, 1.80 (4 x s, 12H, C(O)C*H*$_3$), 1.78 (dd, 1H, H-3a''), 1.70, 1.58 (2 x s, 6H, C(O)C*H*$_3$), 0.75 (m, 2H, H-b, H-b'), -0.2 (s, 9H, Si(CH$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 170.7, 170.15, 170.10, 169.8, 169.6, 165.5, 165.4, 165.3, 165.2, 137.6, 132.9, 132.8, 132.7, 132.5, 130.2, 130.0, 129.83, 129.77, 129.71, 129.64, 129.62, 129.59, 128.6, 128.3, 128.13, 128.12, 128.0, 127.9, 126.3, 101.6, 101.1, 100.0, 81.1, 76.2, 75.6, 74.1, 73.5, 73.0, 72.4, 69.3, 69.1, 68.2, 67.8, 67.2, 67.1, 66.6, 63.0, 62.3, 59.5, 52.8, 49.2, 45.5, 38.1, 23.1, 21.3, 20.9, 20.7, 20.6, 17.8, -1.6; ESI MS calc'd for $C_{72}H_{81}O_{26}SSiNa$: 1458.4. Found: 1458.4. Anal. calc'd for $C_{72}H_{81}O_{26}SSi$: C 60.20, H 5.68. Found: C 60.01, H 5.87.

2-(Trimethylsilyl)ethyl *S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonate)-(2→3)-(2-*O*-benzoyl-3-thio-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (48)

The trisaccharide **47** (0.201 g; 0.14 mmol) was dissolved in 80% acetic acid solution (10.0 mL) and stirred at 45 °C for a period of 48 hours. After this time, the reaction mixture was concentrated on a rotary evaporator while the bath temperature was maintained below room temperature. The residue was evaporated several times from toluene, then chromatographed on silica gel using a gradient of methanol in

dichloromethane (2-2.5%) as eluent to give the diol **48** (0.153 g; 0.11 mmol; 81%): $[\alpha]_D$ 46 (c 1.2, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 8.11 (m, 2H, Ar), 8.00 (m, 2H, Ar), 7.96 (m, 2H, Ar), 7.90 (m, 2H, Ar), 7.56-7.32 (m, 12H, Ar), 5.60 (dd, 1H, H-3, $J_{3,2} = J_{3,4}$ = 9.2 Hz), 5.56 (ddd, 1H, H-8", $J_{8",7"}$ = 10.1 Hz, $J_{8",9a"}$ = 2.5 Hz, $J_{8",9b"}$ 4.0 = Hz), 5.39 (dd, 1H, H-2, $J_{2,1}$ = 7.8 Hz, $J_{2,3}$ = 9.6 Hz), 5.21 (dd, 1H, H-7", $J_{7",6"}$ = 2.3 Hz), 5.12 (dd, 1H, H-2', $J_{2',1'}$ = 7.6 Hz, $J_{2',3'}$= 11.6 Hz), 5.07 (d, 1H, H-1'), 4.96 (d, 1H, N*H*, $J_{NH,5"}$ = 10.0 Hz), 4.76 (ddd, 1H, H-4", $J_{4",3e"}$ = 4.7 Hz, $J_{4",3a"}$ = 11.7 Hz, $J_{4",5"}$ = 10.3 Hz), 4.63 (m, 2H, H-1, H-6b), 4.37 (m, 2H, H6b, H9a"), 4.25 (dd, 1H, H-4, $J_{4,5}$ = 9.2 Hz), 4.21 (dd, 1H, H-9b", $J_{9b",9a"}$ = 12.9 Hz), 3.84 (m, 1H, H-a), 3.77-3.70 (m. 2H, H-5, H-5"), 3.68-3.64 (m, 4H, OCH_3, H-6"), 3.56-3.45 (m, 3H, H-3', H-5', H-a'), 3.30 (m, 1H, H-4'), 3.26 (m, 1H, H-6a'), 3.14 (m, 1H, H6b'), 2.57 (d, 1H, OH-4', $J_{OH-4',H4'}$ = 4.9 Hz), 2.52 (dd, 1H, H-3e", $J_{3e",3a"}$ = 12.8 Hz), 2.08, 1.93, 1.86 (3 x s, 9H, $C(O)CH_3$), 1.82 (dd, 1H, H-3a"), 1.79, 1.64 (2 x s, 6H, $C(O)CH_3$), 1.55 (m, 1H, OH-6'), 0.79 (m, 2H, H-b, H-b'), -0.18 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7(2C), 170.1, 169.91, 169.87, 169.2, 165.7, 165.4, 165.3, 165.2, 133.1, 133.0 (2C), 132.8, 130.19, 130.17, 130.1, 130.0, 129.75, 129.66, 129.65, 129.5, 128.5, 128.4, 128.3, 128.2, 101.5, 100.1, 81.4, 75.5, 75.3, 74.1, 73.4, 73.0, 71.9, 70.3, 69.2, 69.1, 67.3, 67.2, 66.4, 62.9, 62.7, 62.0, 53.0, 49.2, 47.8, 37.2, 23.14, 21.1, 20.9, 20.7, 20.5, 17.8, -1.6; ESI MS calc'd for $C_{65}H_{77}NO_{26}SSiNa$: 1370.4. Found: 1370.4. Anal. calc'd for $C_{65}H_{77}NO_{26}SSi$: C 57.90, H 5.76. Found: C 57.81, H 5.97.

4-Chlorophenyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-nonulopyranosylonate) (49)

Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-chloro-3,5-dideoxy-*D*-*glycero*-β-*D*-*galacto*-non-2-ulopyranosylonate[130] (2.59 g; 5.1 mmol) was dissolved in dichloromethane (50 mL) and was then washed with saturated sodium bicarbonate solution, water, and dried over sodium sulfate before concentration. To this residue in dry tetrahydrofuran (10 mL) was added an ice cold solution of *p*-chloro-thiophenol (1.47 g; 10.2 mmol; 2.0 eq.) and potassium *tert*-butoxide (1.02 g; 1.80 eq.) in *N,N*-dimethylformamide (7.5 mL). The mixture was allowed to warm to room temperature over a period of one hour, after which time it was partially concentrated (~ 5 mL) and then diluted with dichloromethane (50 mL). This solution was washed with 1M HCl (50 mL), saturated sodium bicarbonate solution and water, then dried over sodium sulfate, filtered, and concentrated. Chromatography of the residue on silica gel using a gradient of methanol in dichloromethane (0→1.5%) as eluent afforded **49** (2.29 g; 3.7 mmol; 73%): $[\alpha]_D$ 17 (c 0.57, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44-7.42 (m, 2H, Ar), 7.33-7.30 (m, 2H, Ar), 5.29 (dd, 1H, H-7, $J_{7,6}$ = 1.9 Hz, $J_{7,8}$ = 7.4 Hz), 5.25 (ddd, 1H, H-8, $J_{8,9a}$ = 2.7 Hz, $J_{8,9b}$ = 5.8 Hz, $J \sim 7$ Hz), 5.16 (d, 1H, N*H*, $J_{5,NH}$ = 10.0 Hz), 4.84 (ddd, 1H, H-4, $J_{4,3eq}$ = 4.7 Hz, $J_{4,3a}$ = 11.8, $J_{4,5}$ = 10.2), 4.35 (dd, 1H, H-9a, $J_{9a,9b}$ = 12.4 Hz), 4.15 (dd, 1H, H-9b), 3.97 (ddd, 1H, H-5, $J \sim J \sim J \sim 10$ Hz), 3.90 (dd, 1H, H-6, $J_{6,5}$ = 10.7 Hz), 3.60 (s, 3H, CO_2CH_3), 2.81 (dd, 1H, H-3eq, $J_{3eq,3ax}$ = 12.9 Hz), 2.14, 2.06, 2.05, 2.03 (4s, 12H, 4xC(O)C*H*$_3$), 2.02 (dd, 1H, H-3ax), 1.86 (s, 3H, C(O)C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 170.6, 170.1, 170.0, 169.9, 167.7, 137.5, 136.5, 129.1, 127.1, 87.3, 74.7, 69.6, 69.5, 67.6, 62.1, 52.8, 49.3, 38.2, 23.2 (2C), 20.9 (2C) 20.8; ESI HRMS calc'd for

$C_{26}H_{32}NO_{12}SClNa$: 640.1226. Found: 640.1225. Anal. calc'd for $C_{26}H_{32}NO_{12}SCl$: C 50.53, H 5.22, N 2.27. Found: C 50.19, H 5.59, N 2.20.

4-Chlorophenyl (methyl 4,7,8,9-tetra-*O*-acetyl-5-amino-5-*tert*-butyloxycarbonyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-nonulopyranosylonate) (50)

The tetraacetate **49** (1.275 g; 2.06 mmol) was dissolved in THF (10-15 mL). Di-*tert*-butyl dicarbonate (0.900 g; 4.13 mmol; 2.0 eq.) and *N,N*-dimethyl-4-amino-pyridine (0.050 g) were added. The mixture was heated at 50 °C for 8 hours. After this time the solution was diluted with an equal volume of methanol and hydrazine acetate (1.138 g; 12.36 mmol; 6.0 eq.) was added. After stirring overnight the mixture was diluted with dichloromethane (100 mL), washed with 1M HCl, saturated sodium bicarbonate solution, and water, then dried over sodium sulfate, filtered, and concentrated. The residue was chromatographed on silica gel using toluene/acetone (15:1) to give **50** (0.929 g; 1.37 mmol; 67%): $[\alpha]_D$ 8 (c 0.77, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46-7.42 (m, 2H, Ar), 7.33-7.29 (m, 2H, Ar), 5.37 (dd, 1H, H-7, $J_{7,6}$ = 1.6 Hz, $J_{7,8}$ = 7.7 Hz), 5.28 (ddd, 1H, H-8, $J_{8,9a}$ = 2.9 Hz, $J_{8,9b}$ = 5.8 Hz), 4.77 (ddd, 1H, H-4, $J_{4,3e}$ = 4.6 Hz, $J_{4,5}$ = 11.1 Hz, $J_{4,3a}$ = 11.1 Hz), 4.35 (dd, 1H, H-9a, $J_{9a,9b}$ = 12.4 Hz), 4.22 (d, 1H, N*H*, $J_{NH,5}$ = 10.5 Hz), 4.14 (dd, 1H, H-9b), 3.84 (dd, 1H, H-6, $J_{6,5}$ = 10.9 Hz), 3.67 (ddd, 1H, H-5), 3.58 (s, 3H, OCH_3), 2.82 (dd, 1H, H-3_{eq}, $J_{3eq,3ax}$ = 12.8 Hz), 2.13, 2.06, 2.04, 2.02 (4s, 12H, 4xC(O)CH_3), 1.97 (dd, 1H, H-3_{ax}), 1.38 (s, 9H, C(CH_3)$_3$; ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7, 170.5, 169.8, 169.6, 167.7, 155.1, 137.5, 136.4, 129.1, 127.2, 87.2, 80.1, 74.9, 69.8, 69.2, 67.7, 62.2, 52.8, 50.5, 38.4, 28.1, 20.9, 20.81, 20.77, 20.72; ESI HRMS calc'd for $C_{29}H_{38}NO_{13}SClNa$: 698.1645. Found: 698.1648. Anal. calc'd for $C_{29}H_{38}NO_{13}SCl$: C 51.52, H 5.66, N 2.07. Found: C 51.31, H 5.77, N 2.00.

4-Chlorophenyl (methyl 5-acetamido-4,7,9-tri-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-nonulopyranosylonate) (51)

Thioglycoside **50** (0.810 g; 1.20 mmol) was dissolved in a solution of 25% trifluoroacetic acid in dichloromethane (8.0 mL) at 0 °C. After stirring for 1 hour at 0 °C and 30 minutes at room temperature, the reaction mixture was diluted with toluene (50 mL) and evaporated to dryness at room temperature. The residue was then combined with dichloromethane (5 mL) and triethylamine (0.332 mL; mmol; 2 eq.) and stirred for ~ 20 minutes before concentrating. The crude product was allowed to sit for three days, during which time migration of the acetate to the amine was periodically monitored by ^{1}H NMR (600 MHz, $CDCl_3$, H-5_{amine} δ 2.50 → H-5_{NHAc} δ 4.16). Chromatography on silica gel using toluene/acetone (3:1) as eluent gave **51** (0.594 g; 1.03 mmol; 86%): $[\alpha]_D$ 59 (c 0.74, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45-7.43 (m, 2H, Ar), 7.34-7.31 (m, 2H, Ar), 5.12 (d, 1H, N*H*, $J_{NH,5}$ = 10.3 Hz), 5.04 (dd, 1H, H-7, $J_{7,6}$ = 2.4 Hz, $J_{7,8}$ = 8.7 Hz), 4.80 (ddd, 1H, H-4, $J_{4,3e}$ = 5.0 Hz, $J_{4,5}$ = 10.5 Hz, $J_{4,3a}$ = 11.6 Hz), 4.16 (ddd, 1H, H-5, $J_{5,6}$ = 10.5 Hz), 4.13-4.08 (m, 1H, N*H*, H-8), 4.01-3.96 (m, 2H, H-9a, H-9b), 3.75 (s, 3H, OCH_3), 3.71 (dd, 1H, H-6), 3.52 (bs, 1H, O*H*-8), 2.86 (dd, 1H, H-3_{eq}, $J_{3eq,3ax}$ = 13.0 Hz), 2.14, 2.11 (2s, 6H, 2xC(O)CH_3) , 2.10 (dd, 1H, H-3_{ax}), 2.04, 1.84 (2s, 6H, 2xC(O)CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 170.8, 170.3, 170.1, 169.3, 138.1, 137.2, 129.6, 126.4, 86.0, 75.1, 69.9, 68.9, 68.2, 64.7, 53.6, 48.7, 37.3, 23.1, 20.94, 20.9, 20.8; ESI HRMS calc'd for $C_{24}H_{30}NO_{11}SClNa$: 598.1120. Found: 598.1125. Anal. calc'd for $C_{24}H_{30}NO_{11}SCl$: C 50.04, H 5.25, N 2.43. Found: C 50.01, H 5.29, N 2.65.

_S_-(5-Acetamido-3,5-dideoxy-D-_glycero_-α-D-_galacto_-non-2-ulopyranosylonic acid)-(2→3)-(3-thio-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2_S_,3_R_)-2-(6-aminohexan-amido)-pent-4-ene-1,3-diol (52)

The trisaccharide **77** (0.115 g; 0.08 mmol) was dissolved in anhydrous tetrahydrofuran (8.0 mL) and added via canula to a solution of sodium (0.090 g) in liquid ammonia at –78 °C. Sodium metal (0.060 g) was added, and the dark blue solution was stirred for one hour, then quenched with methanol (15 mL) and acetic acid (0.50 mL). The mixture was allowed to warm to room temperature and was stirred overnight to allow ammonia to evaporate. The methanolic solution was combined with Iatrobeads (~1 g) and concentrated. The solid material was applied to the top of a column of Iatrobeads, and eluted by application of a linear gradient of methanol-isopropanol-water [(1:1:0)→(41:41:18)]. HPLC purification (Hamilton PRP column; water-methanol gradient) of the material obtained yielded **52** (0.0571 g; 0.07 mmol; 83%): $[\alpha]_D$ 33 (c 1.0, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 5.85 (ddd, 1H, H-4_{sph}, $J_{4,3}$ = 7.0 Hz, $J_{4,5a}$ = 17.1 Hz, $J_{4,5b}$ = 10.4 Hz), 5.32 (bd, 1H, H-$5a_{sph}$), 5.26 (bd, 1H, H-$5b_{sph}$), 4.53 (d, 1H, H-1', $J_{1',2'}$ = 7.2 Hz), 4.47 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 4.24-4.20 (m, 1H, H-3_{sph}), 4.11 (ddd, 1H, H-2_{sph}, $J_{2,1a}$ = 6.7 Hz, $J_{2,1b}$ = 3.6 Hz, $J_{2,3}$ = 3.6 Hz), 4.04 (dd, 1H, H-$1a_{sph}$, $J_{1a,1b}$ = 10.5 Hz), 4.00 (dd, 1H, H-6a, $J_{6a,6b}$ = 12.3 Hz, $J_{6a,5}$ = 2.2 Hz), 3.92 (dd, 1H, H-8", J = 2.5 Hz, J = 6.2 Hz, J = 9.2 Hz), 3.88-3.82 (m, 4H, H-4', H-9a", H-5", H-6b), 3.79 (dd, 1H, H-1b), 3.78-3.75 (m, 1H, H-5'), 3.74-3.70 (m, 2H, H-6a', H-6b'), 3.70-3.63 (m, 3H, H-4, H-4", H-7"), 3.64 (dd, 1H, H-3, $J \sim J \sim$ 8.7 Hz), 3.60-3.56 (m, 3H, H-6", H-5, H-9b"), 3.39 (dd, 1H, H-2', $J_{2',3'}$ = 11.4 Hz), 3.37 (dd, 1H, H-3', $J_{3',4'}$ = 2.5 Hz), 3.32 (dd, 1H, H-2), 3.00-2.96 (m, 2H, CH_2NH_2), 2.80 (dd, 1H, H-$3"_{eq}$, $J_{3eq,3ax}$ = 12.7 Hz, $J_{3"eq,4"}$ = 4.8 Hz), 2.28 (dd, 2H, NC(O)CH_2), 2.02 (s, 3H, NHC(O)CH_3), 1.81 (dd, 1H, H-$3"_{ax}$, $J_{3ax,4"}$ = 11.3 Hz),

1.70-1.58 (m, 4H, 2 x CH_2), 1.41-1.34 (m, 2H, CH_2); ^{13}C NMR (125 MHz, D_2O) δ 177.6, 175.9, 175.4, 137.2, 118.9, 105.0, 103.2, 85.0, 79.1, 78.7, 75.9, 75.7, 75.1, 73.8, 72.82, 72.77, 69.7, 69.5, 69.35 (2C), 69.0, 63.5, 62.1, 60.9, 53.9, 52.5, 51.5, 41.5, 40.2, 36.4, 27.4, 26.0, 25.6, 22.9; ESI HRMS calc'd for $C_{34}H_{59}N_3O_{20}SNa$: 884.3316 Found: 884.3310.

(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-(6-aminohexanamido)-pent-4-ene-1,3-diol (53)

The disaccharide **56** (0.025 g; 45 μmol) was combined with CMP-sialic acid (0.135 g of crude mixture, estimated 1.3 eq.), 0.5 M $MnCl_2$ (100 μL; 10 mM final concentration), 0.5 M $MgCl_2$ (100 μL; 10 mM final concentration), 0.4 M dithiothreitol (37.5 μL; 3 mM final concentration), α(2,3) sialyltransferase[217] (4.75 mL; ~0.1 U/mL), and alkaline phosphatase (8 uL; 8U). The mixture was tumbled gently overnight and then centrifuged at 14,000 rpm for 30 minutes to remove cellular debris. The supernatant was applied directly to a size exclusion column (Biogel P4; 50 x 2 cm). Elution with 5% aqueous methanol afforded **53** (0.031 g; 36 μmol; 80 %): $[\alpha]_D$ 3 (c 0.24, H_2O). ^{1}H NMR (500 MHz, D_2O) δ 5.84 (ddd, 1H, H-4$_{sph}$, $J_{4,5}$ = 17.2 Hz, $J_{4,5}$ = 10.4 Hz, $J_{4,3}$ = 7.0 Hz), 5.31 (ddd, 1H, H-5a$_{sph}$, $J_{5,4}$ = 17.2 Hz, $J_{5,5}$ = 1.2 Hz, $J_{5,3}$ = 1.2 Hz), 5.25 (ddd, 1H, H-5b$_{sph}$, $J_{5,4}$ = 10.4 Hz, $J_{5,5}$ = 1.2 Hz, $J_{5,3}$ = 1.2 Hz), 4.51 (d, 1H, H-1', $J_{1',2'}$ = 7.9 Hz), 4.46 (d, 1H, H-1, $J_{1,2}$ = 7.9 Hz), 4.21 (dd, 1H, $J_{3,4}$ = 7.0 Hz, H-3$_{sph}$, $J_{3,2}$ = 6.6 Hz), 4.12-4.08 (m, 2H, H-2$_{sph}$, H-3'), 4.04 (dd, 1H, H-1a$_{sph}$, $J_{1a,1b}$ = 10.5 Hz, $J_{1a,2}$ = 6.4 Hz), 3.98 (dd, 1H, $J_{6a,6b}$ = 12.3 Hz, $J_{6a,5}$ = 2.2 Hz, H-6a), 3.94 (d, 1H, H-4', $J_{4,3}$ = 3.1 Hz), 3.90-3.73 (m, 5H, H-8", H-5", H-9a", H-6b, H-1b$_{sph}$), 3.71-3.67 (m, 3H, H-4", H-6a', H-6b'), 3.66-3.61 (m, 5H, H-5', H-3, H-4, H-9b", H-6"), 3.59-3.55 (m, 3H, H-5 , H-7", H-2'), 3.34-3.30 (m, 1H, H-2),

2.97 (t, 2H, J = 7.6 Hz, CH_2NH_2), 2.75 (dd, 1H, H-3"eq, $J_{3e,3a}$ = 12.3 Hz, $J_{3e,4}$ = 4.6 Hz), 2.28 (t, 2H, $CH_2C(O)$, J = 7.4 Hz), 2.03 (s, 3H, $C(O)CH_3$), 1.79 (dd, 1H, $J_{3a,3e}$ = 12.1 Hz, H-3"ax, $J_{3a,4}$ = 12.1 Hz), 1.69-1.59 (m, 4H, $CH_2CH_2NH_2$, $CH_2CH_2C(O)$), 1.41-1.34 (m, 2H, CH_2); ^{13}C NMR (125 MHz, D_2O) δ 177.6, 175.9, 174.7, 137.2, 118.9, 103.6, 103.2, 100.7, 79.2, 76.4, 76.1, 75.6, 75.1, 73.8, 73.7, 72.8, 72.7, 70.2, 69.5, 69.2, 69.0, 68.4, 63.5, 61.9, 60.9, 53.8, 52.6, 40.6, 40.2, 36.4, 27.4, 26.0, 25.6, 22.9. ESI HRMS calc'd for $C_{34}H_{60}N_3O_{21}$: 846.37193. Found:846.37177.

_S_-(5-Acetamido-3,5-dideoxy-D-_glycero_-α-D-_galacto_-non-2-ulopyranosylonic acid)-(2→3)-[2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)]-(3-thio-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2_S_,3_R_)-2-(6-aminohexanamido)-pent-4-ene-1,3-diol (54)

UDP-*N*-acetylglucosamine disodium salt (0.022 g; 0.02 mmol; 1.5 eq.) was added to a falcon tube containing **52** (15.2 mg; 18 μmol; 1.0 eq.). Freshly prepared β(1,4) *N*-acetylgalactosaminyl transferase[213] (2.0 mL) in 50 mM HEPES, pH 7.5 containing 10% glycerol (v/v) and UDP *N*-acetylglucosamine 4-epimerase[212] (2.0 mL) in 0.2 M NaCl, 1.0 mM EDTA, 20mM HEPES, 5 mM β-mercaptoethanol, pH 7.0 were added to the mixture, followed by 4.0 μL of 0.5M manganese (II) chloride and 12 μL (12 units) of alkaline phosphatase. The mixture was tumbled a total of 24 hours and then centrifuged for 30 minutes at 14,000 rpm. The supernatant was applied to a SepPak cartridge (5 g) which had been conditioned with methanol (10 mL) followed by 1% ammonia solution (20 mL). After application of the sample, the cartridge was washed with 1% ammonia (25 mL) and eluted with 10% methanol containing 1% ammonia. HPLC of the residue thus obtained (Hamilton PRP-1 semi-preparative column; water-methanol gradient elution) afforded the desired GM_2 analogue **54** (18.3 mg; mmol; 74%): $[\alpha]_D$ 22 (c 0.20, H_2O). ^1H NMR (600 MHz, D_2O) δ 5.85 (ddd, 1H, H-4_{sph}, $J_{4,3}$ = 7.0 Hz, $J_{4,5a}$ = 17.3 Hz,

$J_{4,5b}$ = 10.5 Hz), 5.35-5.30 (m, 1H, H-5a$_{sph}$), 5.28-5.25 (m, 1H, H-5b$_{sph}$), 4.75 (obscured, 1H, H-1'''), 4.53 (d, 1H, H-1', $J_{1',2'}$ = 7.6 Hz), 4.47 (d, 1H, H-1, $J_{1,2}$ = 7.9 Hz), 4.24-4.20 (m, 1H, H-3$_{sph}$), 4.11 (ddd, 1H, H-2$_{sph}$, $J_{2,1a}$ = 6.6 Hz, $J_{2,1b}$ = 3.6 Hz, $J_{2,3}$ = 3.6 Hz), 4.06 (dd, 1H, H-1a$_{sph}$, $J_{1a,1b}$ = 10.6 Hz), 4.01-3.98 (m, 2H, H-4', H-6a), 3.92-3.55 (m, 21H, H-3, H-4, H-5, H-6b, H-5', H-6a', H-6b', H-4", H-5", H-6", H-7", H-8", H-9a", H-9b", H-2"', H-3"', H-4"', H-5"', H-6a"', H-6b"', H-1b$_{sph}$), 3.42 (dd, 1H, H-3', $J_{3',2'}$ = 11.5 Hz, $J_{3',4'}$ = 2.4 Hz), 3.32 (dd, 1H, H-2, $J_{2,3}$ = 9.1 Hz), 3.21 (dd, 1H, H-2'), 3.00-2.97 (m, 2H, CH_2NH_2), 2.80 (dd, 1H, H-3"$_{eq}$, $J_{3eq,3ax}$ = 12.6 Hz, $J_{3"eq,4"}$ = 4.8 Hz), 2.28 (dd, 2H, NC(O)CH_2), 2.09 (s, 3H, NHC(O)CH_3), 2.03 (s, 3H, NHC(O)CH_3), 1.84 (dd, 1H, H-3"$_{ax}$, $J_{3ax,4"}$ = 11.6 Hz), 1.70-1.59 (m, 4H, 2 x CH_2), 1.40-1.35 (m, 2H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.6, 175.8, 175.5, 175.3, 137.2, 118.9, 105.0, 104.1, 103.2, 84.6, 79.2, 77.6, 77.0, 75.72, 75.69, 75.5, 75.0, 73.7, 73.0, 72.7, 71.5, 69.44, 69.37, 69.2, 69.1, 68.6, 63.5, 61.9, 61.6, 60.9, 53.8, 53.4, 52.5, 50.5, 41.5, 40.2, 36.4, 27.4, 25.9, 25.6, 23.5, 22.9; ESI HRMS calc'd for $C_{42}H_{72}N_4O_{25}SNa$: 1065.428417. Found: 1065.428462.

(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)-[2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)]-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-(6-aminohexan-amido)-pent-4-ene-1,3-diol (55)

UDP-*N*-acetylglucosamine disodium salt (15 mg; 0.0180 μmol; 1.2 eq.) was added to a Falcon tube containing **53** (23.4 mg; 28.0 μmol; 1.0 eq.). Freshly prepared *N*-acetylgalactosaminyl transferase[213] (1.5 mL, ~1.5U) in 50 mM HEPES, pH 7.5 containing 10% glycerol (v/v) and UDP *N*-acetylglucosamine 4-epimerase[212] (1.5 mL, ~1.5 U) in 0.2 M NaCl, 1.0 mM EDTA, 20mM HEPES, 5 mM β-mercaptoethanol, pH 7.0 were added to the mixture, followed by 75 μL of 0.5M $MnCl_2$ and alkaline

phosphatase (10 μL; 10 U). The mixture was tumbled overnight and then centrifuged for 30 minutes at 14,000 rpm. The supernatant was applied to a tC18 SepPak (5 g), washed with water and eluted with water-methanol. The residue thus obtained was applied to a Biogel P4 column in 5% methanol (50 cm X 2 cm) and eluted at 0.4 mL min^{-1} to yield **55** (23 mg; 22 μmol; 79%): $[\alpha]_D$ 27 (c 0.24, H_2O). ^1H NMR (600 MHz, D_2O) δ 5.85 (ddd, 1H, $J_{4,5a}$ = 17.3 Hz, $J_{4,5b}$ = 10.4 Hz, $J_{4,3}$ = 6.9 Hz, H-4_{sph}), 5.32 (d, 1H, , H-$5a_{sph}$, $J_{5a,4}$ = 17.2 Hz), 5.27 (d, 1H, H-$5b_{sph}$, $J_{5,4}$ = 10.4 Hz), 4.73 (d, 1H, H-1''', $J_{1''',2'''}$ = 7.3 Hz), 4.52 (d, 1H, H-1', $J_{1',2'}$ = 7.9 Hz), 4.48 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 4.23 (dd, 1H, H-3_{sph}, $J_{3,4}$ = 7.0 Hz, $J_{3,2}$ = 7.0 Hz), 4.14 (dd, 1H, H-3', $J_{3',2'}$ = 9.8 Hz, $J_{3',4'}$ = 3.0 Hz), 4.12-4.09 (m, 2H, H-4', H-2_{sph}), 4.06 (dd, 1H, H-$1a_{sph}$, $J_{1a,1b}$ = 10.4 Hz, $J_{1a,2}$ = 6.2 Hz), 3.99 (dd, 1H, H-6a, $J_{6a,6b}$ = 12.2 Hz, $J_{6a,5}$ = 1.9 Hz), 3.93-3.90 (m, 2H, H-4''', H-2''') 3.87 (dd, 1H, $J_{9a,9b}$ = 12.0 Hz, $J_{9a,8}$ = 2.2 Hz, H-9a"), 3.83-3.58 (m, 17H, H-5", H-6b , H-$1b_{sph}$, H-4", H-5', H-6a', H-6b', H-5''', H-6a''', H-6b''', H-8", H-3''', H-3, H-9b", H-4, H-7", H-5), 3.49 (dd, 1H, H-6", $J_{6",5"}$ = 10.1 Hz, $J_{6",7"}$ = 2.1 Hz), 3.36 (dd, 1H, H-2', $J_{2',3'}$ = 9.5 Hz, $J_{2',1'}$ = 7.8 Hz), 3.32 (dd, 1H, H-2, $J_{2,3}$ = 9.2 Hz, $J_{2,1}$ = 8.1 Hz), 2.98 (t, 2H, J = 7.6 Hz, CH_2NH_2), 2.66 (dd, 1H, $J_{3e,3a}$ = 12.6 Hz, $J_{3e,4}$ = 4.3 Hz, H-3"eq), 2.28 (t, J = 7.3 Hz, $CH_2C(O)$), 2.03, 2.01 (2 x s, 6H, $C(O)CH_3$), 1.92 (dd, 1H, $J_{3a,3e}$ = 11.9 Hz, $J_{3a,4}$ = 11.9 Hz, H-3"ax) 1.69-1.60 (m, 4H, $CH_2CH_2NH_2$, $CH_2CH_2C(O)$), 1.40-1.36 (m, 2H, CH_2); ^{13}C NMR (125 MHz, D_2O) δ 177.6, 175.9, 175.7, 174.9, 137.2, 118.9, 103.6, 103.5, 103.2, 102.5, 79.5, 78.0, 75.6 (2 C), 75.2, 75.1, 74.9, 74.0, 73.6, 73.1, 72.7, 72.1, 70.9, 69.6, 69.5, 68.9, 68.7, 63.7, 62.0, 61.5, 61.0, 53.8, 53.2, 52.5, 40.2, 37.9, 36.4, 27.4, 26.0, 25.6, 23.5, 22.9. ESI HRMS calc'd for $C_{42}H_{73}N_4O_{26}$: 1049.45131. Found:1049.45134.

β-D-Galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-(6-aminohexanamido)-pent-4-ene-1,3-diol (ammonium acetate salt) (56)

2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-azido-pent-4-ene-1,3-diol[166] (0.200 g; 0.150 mmol) was dissolved in 90% aqueous pyridine (10.0 mL) and triphenylphosphine (0.100 g; 0.380 mmol; 2.5 eq.) was added. After stirring for 8 hrs, 6-*N*-trifluoracetamido *p*-nitrophenyl caproate[216] (10 eq.) was added, and stirring was continued overnight. The mixture was concentrated, applied to a short plug of silica gel in 2:1 hexanes-ethyl acetate, and eluted with a gradient of the same mixture (3:2→1:1). The material obtained was deprotected by stirring with a catalytic amount of sodium methoxide in methanol (5.0 mL) at 45 °C overnight. After cooling to room temperature, the solution was neutralized by addition of acetic acid, concentrated, suspended in water-methanol (95:5) and filtered a second time. The solution was then applied to a SepPak (5 g) which had been preconditioned with methanol and water containing ~2% ammonium hydroxide and eluted with a gradient of water and methanol containing the same. Concentration of the eluent afforded **56** (0.0498 g; 0.0900 mmol; 58%). A portion of the material was purified further for analysis (Hamilton PRP-1 semi-preparative HPLC column, water-methanol gradient): $[\alpha]_D$ 5 (c 0.40, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 5.85 (ddd, 1H, H-4$_{sph}$), 5.32 (bd, 1H, $J_{5,4}$ = 17.2 Hz, H-5a$_{sph}$, $J_{4,5a}$ = 17.2 Hz, $J_{4,5b}$ = 10.4 Hz, $J_{4,3}$ = 7.0 Hz), 5.27 (bd, 1H, H-5a$_{sph}$, $J_{5,4}$ = 10.4 Hz), 4.48 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 4.44 (d, 1H, H-1', $J_{1',2'}$ = 7.8 Hz), 4.22 (dd, 1H, H-3$_{sph}$, $J_{3,2}$ = 7.0 Hz), 4.11 (ddd, 1H, H-2$_{sph}$, $J_{2,1a}$ = 6.4 Hz, $J_{2,1b}$ = 3.6 Hz), 4.06 (dd, 1H, H-1a$_{sph}$, $J_{1a,1b}$ = 10.6 Hz) 3.98 (dd, 1H, H-6a, $J_{6a,6b}$ = 12.3 Hz, $J_{6a,5}$ = 2.2 Hz), 3.93 (d, 1H, H-4', $J_{4',3'}$ = 3.4 Hz), 3.86-3.69 (m, 5H, H-6b, H-1b$_{sph}$, H-6a', H-6b', H-5'), 3.67-3.62 (m, 3H, H-3', H-3, H-4), 3.62-3.57 (m, 1H, , H-5), 3.54 (dd,

1H, $J_{2,3}$ = 9.9 Hz, $J_{2,1}$ = 7.9 Hz, H-2'), 3.35-3.32 (m, 1H, H-2), 2.98-2.95 (m, 2H, CH_2NH_2), 2.30-2.36 (m, 2H, *J*, $CH_2C(O)N$), 1.91 (s, 3H, $C(O)CH_3$), 1.68-1.58 (m, 4H, $CH_2CH_2NH_2$, $CH_2CH_2C(O)$), 1.40-1.34 (m, 2H, CH_2); ^{13}C NMR (125 MHz, D_2O) δ 177.6, 137.2, 119.0, 103.8, 103.2, 79.4, 76.3, 75.7, 75.1, 73.7, 73.4, 72.8, 71.8, 69.5, 61.9, 60.9, 53.8, 40.2, 36.4, 27.5, 26.0, 25.6, 24.2 ESI HRMS calc'd for $C_{23}H_{43}N_2O_{13}$: 555.2760. Found: 555.2763.

β-D-Glucopyranosyl-(1→1)-(2*S*,3*R*)-2-(6-aminohexan-amido)-pent-4-ene-1,3-diol (57)

Glucoside **72** (0.068 g; 0.08 mmol) in tetrahydrofuran (6 mL) was added to a solution of sodium (0.090 g) in ammonia (50 mL) at –78 °C. After one hour, methanol (5 mL) and acetic acid (0.5 mL) were added, and ammonia was removed by evaporation with a stream of argon. The residue was concentrated and dissolved in water, then applied to a SepPak cartridge (5g) that had been preconditioned with methanol followed by 1% ammonia. After washing with 1% ammonia, the product was eluted with 20% methanol containing the same and concentrated. The residue was purified by HPLC (Hamilton PRP-1 column, water-methanol gradient) to afford **57** (0.026 g; 0.07 mmol; 85%): $[\alpha]_D$ – 1 (c 0.44, CHCl3); ^1H NMR (600 MHz, CD_3OD, δ) 5.87 (ddd, 1H, H-4_{sph}, *J* = 6.6 Hz, *J* = 10.4 Hz, *J* = 17.1 Hz), 5.27 (ddd, 1H, H-$5a_{sph}$, *J* = 1.5 Hz, *J* = 1.6 Hz, *J* = 17.1 Hz), 5.14 (ddd, 1H, H-$5b_{sph}$, *J* = 1.2 Hz, *J* = 1.6 Hz, *J* = 10.4 Hz), 4.26 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 4.16-4.13 (m, 1H, H-3_{sph}), 4.10 (dd, 1H, H-$1a_{sph}$, $J_{1a,1b}$ = 10.2 Hz, $J_{1a,2}$ = 5.7 Hz), 4.04 (ddd, 1H, H-2_{sph}, $J_{2,1b}$ = 3.4 Hz, $J_{2,3}$ = 7.4 Hz), 3.87 (dd, 1H, H-6a, $J_{6a,5}$ = 1.7 Hz, $J_{6a,6b}$ = 11.8 Hz), 3.68-3.64 (m, 1H, H-6b), 3.65 (dd, 1H, H-$1b_{sph}$), 3.37-3.30 (m, 1H, H-3), 3.28-3.25 (m, 2H, H-4, H-5), 3.18 (dd, 1H, H-2), 2.89 (m, 2H, CH_2), 2.23 (m, 2H,

CH_2), 1.70-1.60 (m, 4H, CH_2, CH_2), 1.40 (m, 2H, CH_2); ^{13}C NMR (125 MHz, CD_3OD) δ 175.8, 139.5, 117.0, 104.6, 78.01, 77.95, 75.2, 73.3, 71.6, 69.6, 62.6, 54.7, 40.5, 36.7, 28.3, 26.9, 26.2; ESI HRMS calc'd for $C_{17}H_{33}N_2O_8$: 393.22314. Found: 393.22307.

Allyl *S*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)-3-thio-β-D-galactopyranoside (58)

Allyl glycoside **32** (0.102 g; 0.11 mmol) was dissolved in 80% acetic acid (10 mL) and heated overnight at 45 °C. After concentrating, the residue was evaporated from toluene (3 x 15 mL) and suspended in dry methanol (5 mL). A small piece of sodium was added, and the mixture was stirred at room temperature for 36 hours (higher temperatures resulted in loss of the *N*-acetamido function). After addition of several drops of water, the reaction temperature was increased to 40 °C and stirring was continued overnight. Neutralization with acetic acid followed by concentration afforded a solid which was loaded onto a SepPak cartridge (5 g) in a minimum volume of water. Elution with water afforded **58** (0.049 g; 0.09 mmol; 81%): $[\alpha]_D$ 50 (*c* 0.68, MeOH). ^1H NMR (500 MHz, D_2O) δ 6.04-5.96 (m, 1H, H-b), 5.42-5.37 (m, 1H, H-c), 5.30-5.27 (m, 1H, H-d), 4.53 (d, 1H, H-1, $J_{1,2}$ = 7.3 Hz), 4.43-4.39 (m, 1H, H-a), 4.26-4.22 (m, 1H, H-a'), 3.93-3.82 (m, 4H, H-8', H-9a', H-4, H-5'), 3.75-3.71 (m, 3H, H-5, H-6a, H-6b), 3.70-3.62 (m, 2H, H-4', H-9b'), 3.60-3.56 (m, 2H, H-6', H-7'), 3.39 (dd, 1H, H-2, $J_{2,3}$ = 11.4 Hz), 3.35 (dd, 1H, H-3, $J_{3,4}$ = 2.7 Hz), 2.81 (dd, 1H, H-3'$_{eq}$, $J_{3eq,4}$ = 4.8 Hz, $J_{3eq,3ax}$ = 12.7 Hz), 2.03 (s, 3H, NHC(O)CH_3), 1.82 (dd, 1H, H-3'$_{ax}$, $J_{3ax,4}$ = 11.7 Hz); ^{13}C NMR (125 MHz, D_2O) δ 175.9, 175.4, 134.3, 119.6, 104.0, 85.1, 78.3, 75.8, 72.8, 71.4, 69.7, 69.4, 69.1, 69.0, 63.4, 62.1, 52.5, 51.8, 41.6, 22.9; ESI HRMS calc'd for $C_{20}H_{32}NO_{13}S$: 526.1589. Found: 526.1585.

Allyl (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)-β-D-galactopyranoside (59)

Allyl β-D-galactopyranoside (0.020 g; 0.09 mmol) was combined with crude CMP-Neu5Ac (crude mass 290 mg; estimate 0.10 mmol; 1.1 eq.). α(2,3) Sialyltransferase (50 mM HEPES, pH 7.5, 10% glycerol (v/v); 0.4 U/mL; 1.353 mL), buffer (50 mM HEPES, pH 7.5, 10% glycerol (v/v); 1.5 mL) $MnCl_2$ and $MgCl_2$ (60 μL of a 0.5M solution; final conc. 10 mM), dithiothreitol (38 μL, 0.4M; final conc. 3 mM) and alkaline phosphatase (3 μL; 3U) were added and the mixture was tumbled overnight. After centrifugation (30 min at 14,000 rpm) the supernatant was loaded onto a Biogel P2 column (2.5 x 30 cm) and eluted with water. Final purification by reverse phase HPLC (Beckman C18 column, water-methanol gradient) afforded **59** (0.0311 g; 0.06 mmol; 67%): $[\alpha]_D$ 0 (*c* 0.40, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 5.98 (dddd, 1H, H-b, $J = J = 5.9$ Hz, $J = 10.6$ Hz, $J \sim 17$ Hz), 5.37 (bd, 1H, H-c, $J = 17$ Hz), 5.26 (bd, 1H, H-d, $J = 10.6$ Hz), 4.50 (d, 1H, H-1, $J_{1,2} = 8.0$ Hz), 4.38 (dd, 1H, H-a, $J = 5.7$ Hz, $J = 12.6$ Hz), 4.21 (dd, 1H, H-a', $J = 6.5$ Hz, $J = 12.6$ Hz), 4.07 (dd, 1H, H-3, $J_{3,2} = 9.8$ Hz, $J_{3,4} = 3.1$ Hz), 3.93 (d, 1H, H-4, $J_{3,4} = 3.1$ Hz), 3.88-3.83 (m, 2H, H-8', H-9a'), 3.82 (dd, 1H, H-5', $J_{5',6'} = J_{5',6'} = 10.2$ Hz), 3.77-3.71 (m, 2H, H-6a, H-6b), 3.70-3.57 (m, 5H, H-4', H-5, H-6', H-9b', H-7'), 3.55 (dd, 1H, H-2), 2.74 (dd, 1H, H-3'$_{eq}$, $J_{3eq',4'} = 4.5$ Hz, $J_{3eq',3ax'} = 12.4$ Hz), 2.02 (s, 3H, C(O)CH_3), 1.78 (dd,1H, H-3'$_{eq}$, $J_{3ax',4'} = 12.0$ Hz); ^{13}C NMR (125 MHz, D_2O) δ 175.9, 174.8, 134.3. 119.7, 102.5, 100.7, 76.8, 75.8, 73.7, 72.7, 71.4, 70.0, 69.2, 69.0, 68.4, 63.5, 61.8, 52.6, 40.5, 22.9; ESI HRMS calc'd for $C_{20}H_{32}NO_{14}$: 510.1817. Found: 510.1812.

(2-Trimethylsilyl)ethyl *S*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→6)-(6-thio-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (60)

The trisaccharide **8** (0.045 g; 38 μmol) was dissolved in methanol (3.0 mL) and a small piece of sodium was added. The mixture was stirred for 48 hours and then water (0.30 mL) was added and the temperature was increased to 45 °C for 14 hours. The mixture was neutralized with acetic acid, concentrated, and redissolved in water. Purification by SepPak (water/methanol) followed by HPLC (Hamilton PRP-1 semi-preparative column; water-methanol gradient elution) gave the trisaccharide **60** (0.025 g; 33 μmol; 88%): $[\alpha]_D$ 20 (c 0.36, MeOH); ^{1}H NMR (600 MHz, D_2O) δ 4.49 (d, 1H, H-1, $J_{1,2}$ = 8.1 Hz), 4.42 (d, 1H, H-1', $J_{1',2'}$ = 7.9 Hz), 4.04 (ddd, 1H, H-a, J = 5.2 Hz, J = 10.0 Hz, J = 12.6 Hz), 4.01 (bd, 1H, $J_{4',3'}$ = 3.2 Hz), 3.97 (dd, 1H, H-6a, $J_{6a,5}$ = 2.3 Hz, $J_{6a,6b}$ = 12.4 Hz), 3.87 (dd, 1H, H-9a", $J_{9a",8"}$ = 2.4 Hz, $J_{9a",9b"}$ = 12.0 Hz), 3.84-3.74 (m, 5H, H-5", H-8", H-6b, H-5', H-a'), 3.69 (ddd, 1H, H-4", $J_{4",3e"}$ = 4.7 Hz), $J_{4",3a"}$ = 11.3 Hz, $J_{4",5"}$ = 10.1 Hz), 3.66-3.61 (m, 4H, H-3', H-3, H-4, H-9b"), 3.60-3.56 (m, 3H, H-5, H-6", H-7"), 3.50 (dd, 1H, H-2', $J_{2',3'}$ = 10.0 Hz), 3.36-3.30 (m, 1H, H-2), 2.97 (dd, H-6a', $J_{6a',5'}$ = 7.3 Hz, $J_{6a',6b'}$ = 14.3 Hz), 2.94 (dd, H-6a', $J_{6b',5'}$ = 6.6 Hz), 2.81 (dd, 1H, H-3e", $J_{3e",3a"}$ = 12.7 Hz), 2.03 (s, 3H, NC(O)CH_3), 1.78 (dd, 1H, H-3a"), 1.08 (ddd, 1H, H-b, J = 5.5 Hz, J = 12.8 Hz, J = 12.6 Hz), 0.98 (ddd, 1H, H-b', J = 5.2 Hz, J = 12.8 Hz, J = 12.6 Hz), -0.02 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (125 MHz, D_2O) δ 175.7, 174.7, 103.7, 102.1, 86.7, 79.8, 75.7, 75.6, 75.3, 75.0, 73.7, 73.4, 73.1, 71.6, 69.9, 69.4, 69.3, 68.9, 63.4, 61.1, 52.5, 41.7, 30.4, 22.9, 18.5, -1.6; ESI HRMS calc'd for $C_{28}H_{51}NO_{18}SSiNa$: 772.2488. Found: 772.2490.

_S_-(Methyl 5-acetamido-4,7,8,9-tetra-_O_-acetyl-3,5-dideoxy-D-_glycero_-α-D-_galacto_-non-2-ulopyranosylonate)-(2→6)-(2,3-di-_O_-acetyl-6-thio-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-_O_-acetyl-β-D-glucopyranosyl-(1→1)-(2_S_, 3_R_)-2-azido-3-_O_-benzoyl-pent-4-ene-1,3-diol (63)

(2*S*, 3*R*)-2-azido-3-*O*-benzoyl-4-pentene-1,3-diol[166] (0.020 g; 0.08 mmol), the glycosyl trichloroacetimidate[115] (0.120 g; 0.10 mmol; 1.25 eq.), and Drierite (0.170 g) were combined under an argon atmosphere in freshly distilled dichloromethane (1.5 mL). The mixture was stirred for 45 minutes and then cooled to –10 °C prior to addition of boron trifluoride-diethyl etherate (30 µl; 0.23 mmol; 2.4 eq.). After 1 hour, the temperature was increased to 0 °C for 50 minutes, and then to room temperature for a further 30 minutes. The reaction mixture was diluted with dichloromethane (20 mL), washed with saturated $NaHCO_3$ solution (20 mL) followed by water (20 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated. Column chromatography of the residue in methanol/dichloromethane (3:97) afforded the glycosyl-sphingosine analogue **63** (0.075 g; 0.06 mmol; 71 %): $[\alpha]_D$ –19 (c 0.50, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 (m, 2H, Ar), 7.56 (m, 1H, Ar), 7.44 (m, 2H, Ar), 5.92 (m, 1H, H-4$_{sph}$), 5.64 (dd, 1H, H-3$_{sph}$, J = 4.0 Hz, J = 7.0 Hz), 5.60 (d, 1H, H-4', $J_{4',3'}$ = 3.0 Hz), 5.44 (bd, 1H, H-5a$_{sph}$, $J_{5a,4}$ = 17.3 Hz), 5.39 (bd, 1H, H-5b$_{sph}$, $J_{5b,4}$ = 10.5 Hz), 5.29 (d, 1H, H-7", J = 11.4 Hz), 5.19 (m, 1H, H-8"), 5.17 (dd, 1H, H-3, $J_{3,2}$ = $J_{3,4}$ = 9.4 Hz), 5.11 (d, 1H, N*H*Ac, $J_{NH,5"}$ = 9.2 Hz), 5.02 (m, 2H, H-2', H-3'), 4.96 (dd, 1H, H-2, $J_{2,1}$ = 8.0 Hz), 4.93 (m, 1H, H-4"), 4.69 (d, 1H, H-1', $J_{1',2'}$ = 7.3 Hz), 4.50 (d, 1H, H-1), 4.47 (dd, 1H, H-6a, $J_{6a,5}$ = 3.9 Hz, $J_{6a,6b}$ = 12.6 Hz), 4.26 (dd, 1H, H-9a", $J_{9a,8}$ = Hz, $J_{9a,9b}$ = Hz), 4.15 (dd, 1H, H-9b", $J_{9b",8"}$ = 4.4 Hz), 4.10 (dd, 1H, H-6b, $J_{6b,5}$ = 6.6 Hz), 3.97 (m, 1H, H-2$_{sph}$), 3.92-3.84 (m, 5H, H-1a$_{sph}$, H-4, H-5', H-5", H-6"), 3.83 (s, 3H, OCH_3), 3.63 (ddd, 1H, H-5, $J_{5,4}$ = 2.0 Hz), 3.59 (dd, 1H, H-1b$_{sph}$, $J_{1b,1a}$ = 10.5 Hz, $J_{1b,2}$ = 6.4), 2.78 (dd, 1H, H-6a', $J_{6a',5'}$ = 6.9 Hz, $J_{6a',6b'}$ = 14.4 Hz), 2.70 (dd, 1H, H-3"eq, $J_{3e",3a"}$ = 12.8 Hz, $J_{3e",4"}$ = 4.7 Hz), 2.52 (dd, 1H, H-6b', $J_{6b',5'}$ = 7.7 Hz), 2.18, 2.16, 2.11, 2.10, 2.03, 2.02, 2.014, 2.009, 2.001 (9 x s,

27H, C(O)CH_3), 1.96 (dd, 1H, H-3ax, $J_{3a'',4''}$ = 12.8 Hz), 1.91, 1.87 (2 x s, 6H, C(O)CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7, 170.5, 170.3, 170.2, 169.94, 169.88 (2C), 169.74, 169.6, 169.5, 169.1, 167.8, 164.9, 133.2, 131.2, 129.7, 129.6, 128.4, 120.7, 100.4 , 100.1 , 84.2, 75.7, 74.6, 73.8, 73.3, 72.49, 72.45, 71.5, 71.3, 69.3, 69.1, 67,8, 67.7, 66.7, 63.2, 62.3, 62.2, 53.3, 49.7, 44.1, 29.9, 23.3, 21.2, 21.1, 20.92, 20.85 (multiple C), 20.81, 20.78 (multiple C). ESI MS calc'd for $C_{56}H_{72}N_4O_{30}SNa$: 1335. Found: 1335.

_S_-(Methyl 5-acetamido-4,7,8,9-tetra-_O_-acetyl-3,5-dideoxy-D-_glycero_-α-D-_galacto_-non-2-ulopyranosylonate)-(2→6)-(2,3-di-_O_-acetyl-6-thio-β-D-galactopyranosyl)-(1→4)-_O_-2,3,6-tri-_O_-acetyl-β-D-glucopyranosyl-(1→1)-(2_S_, 3_R_)-3-_O_-benzoyl-2-octadecylamido-pent-4-ene-1,3-diol (64)

Compound **63** (0.0806 g; 0.06 mmol) and triphenylphosphine (0.032 g; 0.12 mmol; 2 eq.) were combined in pyridine/water (10:1) (3.0 mL) and heated at 50 °C. After four hours, *N*-(octadecanoyloxy)-succinimide (0.117 g; 0.31 mmol; 5 eq.) was added, and heating was continued for 4-5 hours. The solution was then cooled and concentrated. The residue was chromatographed on silica gel using a gradient of methanol in dichloromethane (0 to 4%) to afford the title compound (0.084 g; 0.05 mmol; 88%): $[\alpha]_D$ –16 (c 1.0 , $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.00 (m, 2H, Ar), 7.54 (m, 1H, Ar), 7.42 (m, 2H, Ar), 5.88 (ddd, 1H, H-4$_{sph}$, $J_{4,3}$ = 6.3 Hz, $J_{4,5a}$ = 17.0 Hz, $J_{4,5b}$ = 10.6 Hz), 5.78 (d, 1H, NH_{sph}, $J_{NH,H2}$ = 9.0 Hz), 5.60 (ddd, 1H, H-3$_{sph}$, $J_{3,5a}$ = $J_{3,2}$ = 1.2 Hz), 5.40 (dd, 1H, H-4', $J_{4',3'}$ = 3.2 Hz, $J_{4',5'}$ = 1.0 Hz), 5.37 (ddd, 1H, H-5a$_{sph}$, $J_{5a,5b}$ = 1.2 Hz), 5.30-5.26 (m, 2H, H-5b$_{sph}$, H-7''), 5.20-5.14 (m, 2H, H-3, H-8''), 5.12 (d, 1H, N*H*Ac, $J_{NH,5''}$ = 8.8 Hz), 5.02 (dd, 1H, H-2', $J_{2',1'}$ = 7.8 Hz, $J_{2',3'}$ = 10.6 Hz), 4.97 (dd, 1H, H-3'), 4.93 (m, 1H, H-4''), 4.90 (dd, 1H, H-2, $J_{2,1}$ = 7.9 Hz, $J_{2,3}$ = 9.7 Hz), 4.64 (d, 1H, H-1'), 4.50 (m, 1H, H-3$_{sph}$), 4.43 (d, 1H, H-1), 4.38 (dd, 1H, H-6a, $J_{6a,5}$ = 2.2 Hz, $J_{6a,6b}$ = 11.9 Hz), 4.26

(dd, 1H, H-9a", $J_{9a",8"}$ = 3.0 Hz, $J_{9a",9b"}$ = 12.6 Hz), 4.13 (dd, 1H, H-9b", $J_{9b",8"}$ = 4.4 Hz), 4.02 (dd, 1H, H-6b, $J_{6b,5}$ = 6.4 Hz), 3.92-3.81 (m, 7H, H-1a$_{sph}$, H-4, H-5', H-5", OCH_3), 3.63 (dd, 1H, H-1b$_{sph}$, $J_{1b,2}$ = 4.9 Hz, $J_{1b,1a}$ = 10.2 Hz), 3.59 (ddd, 1H, H-5, $J_{5,4}$ = 8.7 Hz, $J_{5,6b}$ = 8.8 Hz), 2.78 (dd, 1H, H-6a', $J_{6a',5'}$ = 7.1 Hz, $J_{6a',6b'}$ = 14.5 Hz), 2.70 (dd, 1H, H-3"eq., $J_{3"eq,3"ax}$ = 12.8 Hz, $J_{3"eq,4"}$ = 4.7 Hz), 2.52 (dd, 1H, H-6b', $J_{6b',5'}$ = 7.5 Hz), 2.16, 2.15 (2 x s, 6H, 2 x C(O)CH_3), 2.13 (m, 2H, CH_{2cer}), 2.10, 2.03, 2.012, 2.008, 2.006, 1.998 (6 x s, 18H, 6 x C(O)CH_3), 1.96 (dd, 1H, H-3"ax, $J_{3"ax,4"}$ = 11.8 Hz), 1.91 (s, 6H, 2 x C(O)CH_3), 1.87 (s, 3H, C(O)CH_3), 1.66-1.54 (m, 4H, 2 x CH_{2cer}), 1.30-1.20 (m, 26H, 13 x CH_{2cer}), 0.85 (t, 3H, CH_{3cer}); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1, 170.8, 170.6, 170.4, 170.2, 170.1, 170.0, 169.9, 169.8, 169.70, 169.69, 169.2, 167.9, 165.1, 133.10, 133.05, 129.9, 129.6, 128.4, 119.0, 100.4 , 100.1 , 84.1, 75.6, 74.1, 73.8, 73.1, 72.8, 72.5, 72.1, 71.8, 71.3, 71.2, 69.2, 69.0, 68.0, 67.6, 67.2, 66.6, 62.2, 62.1, 53.2, 50.4, 49.6, 38.0, 36.7, 31.9, 29.9-29.2 (multiple C), 25.7, 23.2, 22.6, 21.11, 20.97, 20.78, 20.73, 20.72 (multiple C), 20.64, 20.63, 20.53, 20.48, 14.1; ESI MS calc'd for $C_{74}H_{108}N_2O_{31}SNa$: 1575.6. Found: 1575.6; Anal. calc'd for $C_{74}H_{108}N_2O_{31}S$: C 57.20, H 7.01, N 1.80. Found: C 56.86, H 6.99, N 1.69.

_S_-(5-Acetamido-3,5-dideoxy-D-_glycero_-α-D-_galacto_-non-2-ulopyranosylonic acid)-(2→6)-(6-thio-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2_S_, 3_R_)-2-octadecylamido-pent-4-ene-1,3-diol (65)

Trisaccharide **64** (0.027 g; 27 μmol) was combined with dry methanol (3.0 mL) and a small piece of sodium (~5mg) was added. The solution was stirred for 24 hours and then water (300 μL) was added. Stirring was continued for a further 24 hours, then the mixture was neutralized with acidic ion exchange resin, filtered, and concentrated. The residue was loaded onto a preconditioned SepPak cartridge (0.4 g) in 10% methanol,

washed extensively with water and eluted with methanol. Concentration of the eluates gave a residue which was further purified by HPLC (Kromasil C_{18} column, methanol/water gradient) to afford compound **65** (0.015 g; 84%): $[\alpha]_D$ 17 (c 0.71, MeOH); ^{1}H NMR (600 MHz, CD_3OD, δ) 5.86 (ddd, 1H, H-4$_{sph}$, $J_{4,3}$ = 6.8 Hz, $J_{4,5a}$ = 17.2 Hz, $J_{4,5b}$ = 10.4 Hz), 5.27 (ddd, 1H, H-5a$_{sph}$, J = 17.2 Hz, 1.3 Hz, 1.7 Hz), 5.13 (ddd, 1H, H-5b$_{sph}$, J = 10.4 Hz, 1.2 Hz, 1.8 Hz), 4.39 (d, 1H, H-1', $J_{1',2'}$ = 7.5 Hz), 4.30 (d, 1H, H-1, $J_{1,2}$ = 7.9 Hz), 4.19-4.13 (m, 2H, H-1a$_{sph}$, H-3$_{sph}$), 4.02-3.98 (m, 2H, H-4', H-3$_{sph}$), 3.90 (dd, 1H, H-6a, $J_{6a,6b}$ = 12.0 Hz $J_{6a,5}$ = 2.7 Hz), 3.86-3.77 (m, 4H, H-6b, H-9a", H-9b", H-5'), 3.74-3.64 (m, 2H, H-4", H-5"), 3.61-3.56 (m, 3H, H-1$_{sph}$, H-4, H-8"), 3.55-3.40 (m, 6H, H-3, H-3', H-7", H-6", H-2', H-5), 3.32 (dd, 1H, H-2, $J_{2,3}$ = 9.0 Hz), 2.99 (d, 2H, H-6a', H-6b', $J_{6a,6b}$ = 7.1 Hz, 2.89 (dd, 1H, H-3$_{eq}$", $J_{3e,4}$ = 4.8 Hz, $J_{3e,3a}$ = 12.5 Hz), 2.18 (t, 2H, C(O)CH_2, J = 7.5 Hz), 1.99 (s, 3H, NHAc), 1.64 (dd, 1H, H-3$_{ax}$", $J_{3a,4}$ =10.8 Hz), 1.62-1.54 (m, 2H, C(O)CH$_2$CH_2), 1.35-1.25 (m, 28H, alkyl), 0.89 (t, 3H, CH_3, J = 7 Hz); ^{13}C NMR (125 MHz, CD_3OD) δ 176.2, 175.5, 174.8, 139.7, 117.0, 104.7, 104.4, 87.6, 80.5, 76.8, 76.6, 76.4, 76.0, 74.9, 74.7, 73.3, 73.2, 72.4, 70.4, 70.1, 64.8, 64.5, 62.0, 54.7, 54.0, 43.2, 37.2, 33.1, 30.78 (multiple C), 30.75, 30.66, 30.49, 30.47, 30.37, 27.1, 23.7, 22.6, 14.4; ESI HRMS calc'd for $C_{46}H_{82}O_{20}SNa$: 1037.5079. Found: 1037.5080

_S_-(5-Acetamido-3,5-dideoxy-D-_glycero_-α-D-_galacto_-non-2-ulopyranosylonic acid)-(2→6)-(6-thio-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2_S_, 3_R_, 4E)-2-octadecylamido-octadec-4-ene-1,3-diol (66)

The trisaccharide **64** (0.024 g; 0.015 mmol) was dissolved in dichloromethane (4.0 mL) and pentadecene (0.0085 mL; 0.030 mmol; 2.0 eq.). Grubbs' catalyst, 2nd Generation (0.005 g; 5.9 μmol; 0.4 eq.) was added, and the mixture was heated at reflux. After a period of 36 hours, additional pentadecene (1.0 eq.) was added. After stirring for 5 days,

the mixture was chromatographed on a silica gel column using a gradient of acetone in toluene (20→25%) to afford starting material (0.0147 g; 61%) and the target **66** (0.0049 g; 0.003 mmol; 18%): $[\alpha]_D$ – 3 (c 0.22, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.01-7.97 (m, 2H, Ar), 7.56-7.51 (m, 1H, Ar), 7.44-7.40 (m, 2H, Ar), 5.84 (ddd, 1H, H-5_{sph}, $J_{5,4}$ = 15.3 Hz, J = 7.0 Hz, J = 7.0 Hz), 5.74 (d, 1H, N*H*C(O), $J_{NH,H2sph}$ = 9.1 Hz), 5.53 (dd, 1H, H-3_{sph}, $J_{3,2}$ = 7.2 Hz, $J_{3,4}$ = 7.4 Hz), 5.49 (dd, 1H, H-4', $J_{4',5'}$ = 0.8 Hz, $J_{4',3'}$ = 3.0 Hz), 5.45 (dd, 1H, H-4_{sph}), 5.29 (dd, 1H, H-7", J = 1.3 Hz, J = 9.7 Hz), 5.21-5.17 (m, 1H, H-8"), 5.15 (dd, 1H, H-3, $J_{3,4}$ = 9.3 Hz, $J_{3,2}$ = 9.7 Hz), 5.13 (d, 1H, N*H*Ac, $J_{NH,5"}$ = 9.3 Hz), 5.04-4.91 (m, 3H, H-2', H-3', H-4"), 4.90 (dd, 1H, H-2, $J_{2,1}$ = 7.9 Hz), 4.64 (d, 1H, H-1', $J_{1',2'}$ = 7.3 Hz), 4.48-4.42 (m, 2H, H-2_{sph}, H-1), 4.36 (dd, 1H, H-6a, $J_{6a,5}$ = 2.1, $J_{6a,6b}$ = 11.9 Hz), 4.26 (dd, 1H, H-9a", $J_{9a",8"}$ = 3.0 Hz, $J_{9a",9b"}$ = 12.7 Hz), 4.14 (dd, 1H, H-9b", $J_{9b",8"}$ = 4.3 Hz), 4.02-3.96 (m, 2H, H-$1a_{sph}$, H-6b), 3.90-3.81 (m, 7H, H-5", H-5', H-6", H-4, OCH_3), 3.62-3.55 (m, 2H, H-$1b_{sph}$, H-5), 3.47 (bs, 2H, CH_2), 2.78 (dd, 1H, H-6a', $J_{6a',5'}$ = 7.2 Hz, $J_{6a',6b'}$ = 14.5 Hz), 2.71 (dd, 1H, H-3_{eq}", $J_{3e,4"}$ = 4.6 Hz, $J_{3e,3a}$ = 12.7 Hz), 2.52 (dd, 1H, H-6b', $J_{6b",5"}$ = 7.4 Hz), 2.17, 2.16, 2.11, 2.04, 2.02, 2.01, 2.004, 1.995, 1.91, 1.89, 1.88 (11s, 33H, 11xC(O)CH_3), 2.0 (m, 2H, CH_2), 1.97 (m, 1H, H-3_{ax}"), 1.63-1.50 (m, 6H), 1.36-1.18 (m, 50H), 0.90-0.83 (m, 6H, 2xCH_3); ^{13}C NMR (125 MHz, CD_3OD) δ 172.6, 170.8, 170.6, 170.4, 170.2, 170.1, 170.01, 169.95, 169.80, 169.7 (2C), 169.2, 168.0, 165.2, 137.4, 130.0, 129.6, 129.0, 128.4, 124.6, 100.4, 100.1, 84.1, 75.7, 74.1, 73.8, 73.1, 72.5, 72.2, 71.8, 71.2, 69.2, 69.0, 68.0, 67.7, 67.4, 66.7, 62.1, 53.3, 48.8, 36.8, 32.4, 31.9, 29.9-28.9 (multiple C), 25.7, 22.7, 21.2-20.5 (multiple C), 14.1; HRMS calc'd for $C_{87}H_{134}N_2O_{31}SNa$: 1757.8589. Found: 1757.8588.

2,3-Di-*O*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-azido-3-*O*-benzoyl-pent-4-ene-1,3-diol (68)

A suspension of the imidate[199] (0.301 g; 0.61 mmol; 1.28 eq.), (2*S*,3*R*)-2-azido-3-*O*-benzoyl-pent-4-ene-1,3-diol (0.117 g; 0.47 mmol; 1.00 eq.) and powdered 4Å molecular sieves (100 mg) were combined in freshly distilled dichloromethane (10.0 mL) and stirred for 30 minutes under an argon atmosphere at room temperature. The mixture was then cooled to –50 °C and trimethylsilyl trifluoromethanesulfonate (20 μL in 0.5 mL of dichloromethane) was added. The reaction mixture was allowed to warm to room temperature and stirring was continued for six hours before triethylamine was added to neutralize the mixture. After filtration and concentration, the residue was chromatographed on silica gel using hexanes-ethyl acetate (4:1) as eluent to afford **68** (0.248 g; 4.3 mmol; 90%): $[\alpha]_D$ – 59 (c1.52, CHCl3); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09-8.06 (m, 2H, Ar), 7.62-7.57 (m, 1H, Ar), 7.50-7.41 (m, Ar, 4H), 7.37-7.33 (m, 3H, Ar), 5.96 (ddd, 1H, H-4$_{sph}$, J = 7.1 Hz, J = 10.6 Hz, J = 17.2 Hz), 5.67-5.63 (m, 1H, H-3$_{sph}$), 5.52-5.46 (m, 2H, C*H*Ph, H-5$_{sph}$), 5.45-5.40 (m, 1H, H-5$_{sph}$), 5.31 (dd, 1H, H-3, $J_{3,2}$ = $J_{3,4}$ = 9.4 Hz), 5.04 (dd, 1H, H-2, $J_{2,1}$ = 7.8 Hz), 4.29 (dd, 1H, H-6a, $J_{6a,6b}$ = 10.5 Hz, $J_{6a,5}$ = 5.0 Hz), 4.01-3.90 (m, 2H, C-2$_{sph}$, C-1$_{sph}$), 3.75-3.64 (m, 3H, H-4, H-6b, C-1$_{sph}$), 3.53 (ddd, 1H, H-5, J = 5.0 Hz, J = 9.9 Hz, J = 9.9 Hz), 2.10 (s, 3H, C(O)C*H*$_3$) 2.05 (s, 3H, C(O)C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.1, 169.5, 165.0, 136.7, 133.4, 131.4, 129.8, 129.7, 129.2, 128.5, 128.2, 126.1, 120.8, 101.5, 101.0, 78.2, 74.4, 72.1, 71.8, 68.4, 68.2, 66.5, 63.2, 20.8, 20.7; ESI HRMS calc'd for $C_{29}H_{31}N_3O_{10}Na$: 604.1902. Found: 604.1907. Anal. calc'd for $C_{29}H_{31}N_3O_{10}$: C 59.89, H 5.37, N 7.23. Found: C 59.61, H 5.20, N 7.09.

A side product, **(2*S*,3*R*)-1-*O*-Acetyl-2-azido-3-*O*-benzoyl-pent-4-ene-1,3-diol (67)** was isolated (0.007 g; 0.001 mmol; 4%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10-8.06 (m, 2H, Ar), 7.63-7.57 (m, 1H, Ar), 7.51-7.45 (m, 2H, Ar), 6.01-5.91 (m, 1H, H-4), 5.69-5.64 (m, 1H, H-3), 5.54-5.47 (m, 1H, H-5a), 5.45-5.41 (m, 1H, H-5b), 4.28 (dd, 1H, H-1a, $J_{1a,2}$ = 4.6 Hz, $J_{1a,1b}$ = 11.6 Hz), 4.19 (dd, 1H, H-1b, $J_{1b,2}$ = 7.8 Hz), 4.02 (ddd, 1H, H-2, *J* = 4.5 Hz, *J* = 4.5 Hz, *J* = 7.8 Hz), 2.12 (s, 3H, C(O)CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 165.1, 133.5, 131.2, 129.8, 129.4, 128.5, 120.8, 74.3, 62.9, 62.6, 20.7; ESI HRMS calc'd for $C_{14}H_{15}N_3O_4Na$: 312.0955. Found: 312.0953.

2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-azido-3-*O*-benzyl-pent-4-ene-1,3-diol (69)

The diacetate **68** (1.053 g; 1.81 mmol) was dissolved in dry methanol (30 mL) and a small piece of sodium was added. After 3 hours, the mixture was neutralized with acidic cation exchange resin, filtered, and concentrated. The residue was dissolved in anhydrous *N,N*-dimethylformamide (25 mL) and cooled to 0 °C. Sodium hydride (0.434 g of a 60% dispersion in oil; 10.9 mmol; 6 eq.) was added in portions, followed by benzyl bromide (1.3 mL; 10.9 mmol; 6 eq.). After 4 hours methanol was added followed by acidic cation exchange resin. The neutral solution was filtered and concentrated, then redissolved in dichloromethane (100 mL) and washed with water (2 x 60 mL), dried and concentrated. The residue was chromatographed on silica gel with hexanes-ethyl acetate (10:1) as eluent to give **69** (1.136 g; 1.71 mmol; 94%): $[\alpha]_D$ – 43 (c 1.21, CHCl3); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51-7.48 (m, 2H, Ar), 7.41-7.25 (m, 18H, Ar), 5.84 (ddd, 1H, H-4_{sph}, *J* = 7.9 Hz, *J* = 10.4 Hz, *J* = 18.3 Hz), 5.57 (s, 1H, C*H*Ph), 5.44-5.41 (m, 1H, H-$5a_{sph}$), 5.38-5.33 (m, 1H, H-$5b_{sph}$), 4.92 (d, 1H, CH_2Ph, *J* = 11.4 Hz), 4.84 (d, 1H,

CH_2Ph, J = 11.0 Hz), 4.80 (d, 1H, CH_2Ph, J = 11.4 Hz), 4.78 (d, 1H, CH_2Ph, J = 11.0 Hz), 4.62 (d, 1H, CH_2Ph, J = 11.9 Hz), 4.51 (d, 1H, H-1, $J_{1,2}$ = 7.6 Hz), 4.38 (d, 1H, CH_2Ph, J = 11.4 Hz), 4.35 (dd, 1H, H-6a, J = 10.4 Hz, 5.0 Hz), 4.00 (dd, 1H, H-1a$_{sph}$, J = 6.7 Hz, J = 10.0 Hz), 3.95 (dd, 1H, H-3$_{sph}$, J = 5.3 Hz, J = 7.9 Hz), 3.81-3.67 (m, H-6b, H-1b$_{sph}$, H-3, H-4, H-2$_{sph}$), 3.48 (dd, 1H, H-2, $J_{2,3}$ = 8.3 Hz), 3.44-3.37 (m, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.5, 138.3, 137.8, 137.3, 134.3, 128.9, 128.4, 128.34, 128.28, 128.23, 127.99, 127.96, 127.72, 127.68, 127.63, 127.61, 126.0, 120.8, 103.9, 101.2, 82.1, 81.5, 80.9, 79.8, 75.4, 75.1, 70.5, 68.8, 68.7, 66.1, 64.2; ESI HRMS calc'd for $C_{39}H_{41}N_3O_7Na$: 686.2837. Found: 686.2838. Anal. calc'd for $C_{39}H_{41}N_3O_7$: C 70.57, H 6.23, N 6.33. Found: C 70.94, H 6.40, N 6.07.

2,3,6-Tri-*O*-benzyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-azido-3-*O*-benzyl-pent-4-ene-1,3-diol (70)

The azide **69** (0.720 g; 1.08 mmol) was concentrated twice from toluene and the residue was dissolved in tetrahydrofuran (20 mL) containing powdered 3Å molecular sieves. After stirring for twenty minutes, sodium cyanoborohydride (0.681 g; 10.84 mmol; 10 eq.) was added, followed by a crystal of methyl orange. A saturated solution of hydrochloric acid in diethyl ether was added to the mixture, dropwise, until a pink colour persisted. After ninety minutes an additional 3 mL of acid solution was added. After a total of 2.5 hours, the reaction mixture was filtered into a separatory funnel containing a 1:1 mixture of dichloromethane and water (150 mL). The organic layer was removed, dried over sodium sulfate and concentrated. Chromatography of the residue, using hexanes-ethyl acetate (5:1) as eluent afforded **70** (0.685 g; 1.03 mmol; 95%): $[\alpha]_D$ -34.30 (c 0.52, CHCl3); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36-7.24 (m, 24H, Ar), 5.83 (m, 1H,

OCH_2CHCH_2, J = 8.0 Hz, J = 10.4 Hz, J = 17.4 Hz), 5.42-5.39 (m, 1H, OCH_2CHCH_2), 5.35-5.31 (m, 1H, OCH_2CHCH_2), 4.92 (d, 1H, OCH_2Ph, 1H, J = 11.6 Hz), 4.87 (d, 1H, OCH_2Ph, J = 11.3 Hz), 4.74 (d, 1H, OCH_2Ph, J = 11.6 Hz), 4.71 (d, 1H, OCH_2Ph, J = 11.2 Hz), 4.62 (d, 1H, OCH_2Ph, J = 11.8 Hz), 4.59 (d, 1H, OCH_2Ph, J = 12.0 Hz), 4.55 (d, 1H, OCH_2Ph, J = 12.0 Hz), 4.41 (d, 1H, H-1, $J_{1,2}$ = 7.3 Hz), 4.38 (d, 1H, OCH_2Ph, J = 11.8 Hz), 4.02 (dd, 1H, H-6a, $J_{6a,5}$ = 6.7 Hz, $J_{6a,6b}$ = 10.2 Hz), 3.96 (dd, 1H, H-3_{sph}, J = 5.3 Hz, J = 7.9 Hz), 3.78-3.60 (m, 5H, H-4, H-5, H-6b, H-2_{sph}, H-$1a_{sph}$), 3.49-3.40 (m, 3H, H-$1b_{sph}$, H-2, H-3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.6, 138.4, 137.87 (2C), 134.3, 128.5, 128.42, 128.38, 128.37, 128.0, 127.9, 127.8, 127.7, 127.68, 127.67, 127.65, 127.62, 120.7, 103.5, 84.0, 81.7, 79.8, 75.3, 74.8, 74.1, 73.7, 71.6, 70.4, 70.2, 68.4, 64.2; ESI HRMS calc'd for $C_{34}H_{36}O_6Na$: 688.2993. Found: 688.3001. Anal. calc'd for $C_{34}H_{36}O_6$: C 70.36, H 6.51, N 6.31. Found: C 70.46, H 6.68, N 6.06.

2,3,6-Tri-*O*-benzyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-(*N*-carboxybenzyl-6-aminohexan-amido)-3-*O*-benzyl-pent-4-ene-1,3-diol (71)

The azido alcohol **70** (0.201 g; 0.30 mmol) was taken up in pyridine-water (9:1; 10 mL) and triphenylphosphine (160 mg; 0.61 mmol; 2 eq.) was added. The mixture was stirred at 45 °C for 3 hours and then cooled to room temperature. 6-(Benzyloxycarbonylamino)-hexanoic acid succinimidyl ester (0.383 g; 3.5 eq.) was added and the reaction mixture was stirred at 45 °C for a further 2.5 hours, then concentrated. The residue was chromatographed on silica gel using a linear gradient of dichloromethane (15:1 → 12:1) as eluent to yield **71** (0.211 g; 0.24 mmol; 79%): α_D −17.30 (c 0.23, CHCl3); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39-7.31 (m, 25H, Ar), 5.79 (d, 1H, N*H*C(O)CH_2), 5.74 (ddd, 1H, H-4_{sph}, J = 7.9 Hz, J = 10.5 Hz, J = 17.5 Hz), 5.28 (bd, 1H, H-$5a_{sph}$, J = 10.6 Hz), 5.20

(bd, 1H, H-5b$_{sph}$, J = 17.1), 5.09 (s, 2H, CH_2Ph), 4.82 (d, 1H, CH_2Ph, J = 11.9 Hz), 4.80 (d, 1H, CH_2Ph, J = 11.9 Hz), 4.79 (bs, 1H, CH$_2$NH), 4.76 (s, 2H, CH_2Ph), 4.61 (d, 1H, CH_2Ph, J = 12.0 Hz), 4.58 (d, 1H, CH_2Ph, J = 11.9 Hz), 4.54 (d, 1H, CH_2Ph, J = 11.9 Hz), 4.36 (d, 1H, H-1, $J_{1,2}$ = 7.4 Hz), 4.35 (d, 1H, CH_2Ph, J = 11.9 Hz), 4.25 (dd, 1H, H-1a$_{sph}$, J = 4.5 Hz, J = 10.4 Hz), 4.27-4.18 (m, 1H, H-2$_{sph}$), 3.88 (dd, 1H, H-3$_{sph}$, J = J = 7.3 Hz), 3.75 (dd, 1H, H-6a, $J_{6a,5}$ = 4.2 Hz, $J_{6a,6b}$ = 10.3 Hz), 3.71 (dd, 1H, H-6b, $J_{6b,5}$ = 5.1 Hz), 3.67-3.62 (m, 2H, H-1b$_{sph}$, H-4), 3.48 (dd, 1H, H-3, $J_{3,2}$ = $J_{3,4}$ = 9.0 Hz), 3.42 (ddd, H5, $J_{5,4}$ = 9.4 Hz), 3.38 (dd, 1H, H-2), 3.16-3.09 (m, 2H, CH_2NH$_2$), 1.91-1.79 (m, 2H), 1.51-1.37 (m, 4H), 1.22-1.17 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.4, 156.4, 138.5, 138.23, 138.17, 137.7, 136.6, 135.7, 128.53, 128.50, 128.46, 128.45 (2C), 128.3, 128.10, 128.07, 127.96, 127.85, 127.81, 127.78, 127.76, 127.70, 127.6, 119.4, 104.0, 84.2, 81.7, 79.5, 75.3, 74.8, 74.0, 73.7, 71.9, 70.6, 70.2, 68.4, 66.6, 52.0, 40.9, 36.2, 29.6, 26.2, 25.0; ESI HRMS calc'd for $C_{53}H_{62}N_2O_{10}Na$: 909.4297. Found: 909.4298. Anal. calc'd for $C_{53}H_{62}N_2O_{10}$: C 71.77, H 7.04, N 3.16. Found: C 71.73, H 6.71, N 2.96.

2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-(*N*-carboxybenzyl-6-aminohexan-amido)-3-*O*-benzyl-pent-4-ene-1,3-diol (72)

The azide **69** (0.098 g; 0.15 mmol) was dissolved in a mixture of pyridine and water (20:1; 5 mL) and triphenylphosphine (0.078 g; 0.29 mmol; 2.0 eq.) was added. The solution was heated at 50 °C for 4.5 hours and then cooled to room temperature. 6-(Benzyloxycarbonylamino)-hexanoic acid succinimidyl ester (0.107 g; 0.29 mmol; 2.0 eq.) was added to the flask, and after heating overnight at 50 °C the mixture was concentrated. Chromatography on silica gel using a linear gradient of methanol in dichloromethane (5.0→6.2 %) as eluent afforded **72** (0.112 g; 0.13 mmol; 85%): $[\alpha]_D$

-25.09 (c 0.31, CHCl3); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.51-7.48 (m, 2H, Ar), 7.41-7.25 (m, 23H, Ar), 5.75 (ddd, 1H, H-4$_{sph}$, J = 7.9 Hz, J = 10.3 Hz, J = 17.3 Hz), 5.71 (d, 1H, N*H*C(O)CH_2, J = 9.4 Hz), 5.58 (s, 1H, d, C*H*Ph), 5.31-5.27 (m, 1H, H-5a$_{sph}$), 5.23-5.18 (m, 1H, H-5b$_{sph}$), 5.09 (s, 2H, CH_2Ph), 4.94 (d, 1H, CH_2Ph, J = 11.5 Hz), 4.83 (d, 1H, CH_2Ph, J = 11.1 Hz), 4.80 (d, 1H, CH_2Ph, J = 11.5 Hz), 4.76 (bt, 1H, N*H*CO_2Bn), 4.69 (d, 1H, CH_2Ph, J = 11.1 Hz), 4.62 (d, 1H, CH_2Ph, J = 11.9 Hz), 4.45 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 4.35 (d, 1H, CH_2Ph, J = 11.9 Hz), 4.34 (dd, 1H, H-6a, $J_{6a,5}$ = 5.0 Hz, $J_{6a,6b}$ = 10.4 Hz), 4.29 (dd, 1H, H-1a$_{sph}$, $J_{1a,2}$ = 3.9 Hz, $J_{1a,1b}$ = 10.5 Hz), 4.23 (m, 1H, H-2$_{sph}$), 3.85 (dd, 1H, H-3$_{sph}$, $J_{3,2} = J_{3,4}$ = 7.6 Hz), 3.80-3.75 (m, 2H, H-3, H-6b), 3.70 (dd, 1H, H-4, $J_{4,3} = J_{4,5}$ = 9.4 Hz), 3.61 (dd, 1H, H-1b$_{sph}$, $J_{1,2}$ = 3.7 Hz), 3.44 (dd, 1H, H-2, $J_{2,3}$ = 8.7 Hz), 3.39 (ddd, 1H, H-5, $J_{5,6b}$ = 9.6 Hz), 3.16-3.11 (m, 2H, CH_2NHCO_2Bn), 1.89-1.75 (m, 2H, C(O)CH_2), 1.49-1.38 (m, 4H, 2 x CH_2), 1.22-1.15 (m, 2H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.3, 156.4, 138.3, 138.1 (2C), 137.3, 136.6, 135.7, 129.0, 128.51, 128.46, 128.35, 128.33, 128.25, 128.11, 128.06, 128.04, 127.87, 127.84, 127.80, 127.70, 127.66, 126.0, 119.6, 104.4, 101.2, 82.0, 81.7, 81.1, 79.5, 75.4, 75.0, 70.6, 68.8, 68.7, 66.6, 66.1, 51.7, 40.9, 36.2, 29.6, 26.2, 25.0; ESI HRMS calc'd for $C_{53}H_{60}N_2O_{10}Na$: 907.4146. Found: 907.4145. Anal. calc'd for $C_{53}H_{60}N_2O_{10}$: C 71.92, H 6.83, N 3.17. Found: C 71.73, H 6.71, N 2.96.

_S_-(Methyl 5-acetamido-4,7,8,9-tetra-_O_-acetyl-3,5-dideoxy-D-_glycero_-α-D-_galacto_-non-2-ulopyranosylonate)-(2→3)-2-_O_-benzoyl-4,6-_O_-benzylidene-3-thio-D-galactopyranose (73)

Disaccharide **32** (0.950 g; 1.05 mmol), palladium chloride (0.265 g; 1.49 mmol; 1.4 eq.), and sodium acetate (0.360 g; 4.39 mmol; 4.2 eq.) were combined and mixed in 30 mL

acetic acid-water (19:1). The reaction was immersed in an ultrasonic cleaning bath (Branson model B-32) and water temperature was maintained below 30 °C by portionwise addition of ice. After 5 hours, the mixture was filtered through celite, diluted with ethyl acetate (80 mL), washed with an equal volume of water, dried over sodium sulfate, filtered and concentrated. The residue was chromatographed using hexanes-ethyl acetate-acetone (3:3:2) as eluent to yield **73** (0.735 g; 0.85 mmol; 81%) as an inseparable mixture of α and β isomers: ESI HRMS calc'd for $C_{40}H_{47}NO_{18}SNa$: 884.2406. Found: 884.2405.

_S_-(Methyl 5-acetamido-4,7,8,9-tetra-_O_-acetyl-3,5-dideoxy-D-_glycero_-α-D-_galacto_-non-2-ulopyranosylonate)-(2→3)-2-_O_-benzoyl-4,6-_O_-benzylidene-3-thio-D-galactopyranosyl trichloroacetimidate (74)

The hemiacetal 25 (0.560 g; 0.65 mmol) was concentrated from toluene (2x) and dried in vacuo. The residue was then taken up in dichloromethane (10.0 mL) and then trichloroacetonitrile (1.0 mL; 10 mmol; 15.4 eq.) and 1,8-diazabicyclo-[5.4.0]-undec-7-ene (0.050 mL; 0.33 mmol; 0.5 eq.) were added. After two hours, the reaction mixture was concentrated and the residue chromatographed on silica gel with ethyl acetate-hexanes (7:1) containing triethylamine (2%) as eluent to afford **74** (0.458 g; 0.46 mmol; ~70%) and some minor contaminants (<10%). ESI HRMS calc'd for $C_{42}H_{47}N_2O_{18}SCl_3Na$: 1027.1505. Found: 1027.1502.

_S_-(Methyl 5-acetamido-4,7,8,9-tetra-_O_-acetyl-3,5-dideoxy-D-_glycero_-α-D-_galacto_-non-2-ulopyranosylonate)-(2→3)-(2-_O_-benzoyl-4,6-_O_-benzylidene-3-thio-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-_O_-benzyl-β-D-glucopyranosyl-(1→1)-(2_S_,3_R_)-2-azido-3-_O_-benzyl-pent-4-ene-1,3-diol (75)

The acceptor **70** (0.110 g; 0.17 mmol) and imidate **74** (0.250 g; 0.25 mmol; 1.5 eq.) were combined and concentrated from toluene at room temperature. The residue was

dissolved in freshly distilled dichloromethane (12 mL) containing powdered 4Å molecular sieves, and the mixture was stirred under argon for 45 minutes at room temperature before being cooled to 0 °C. Boron trifluoride-diethyl etherate (0.030 mL; 0.24 mmol; 1.5 eq.) was added and the reaction mixture was allowed to warm to room temperature. After 1.5 hours, an additional 0.5 equivalents of boron trifluoride-diethyl etherate was added and stirring was continued for two hours before the mixture was made basic with triethylamine, filtered, and concentrated. Chromatography of the residue on silica gel with a gradient of hexanes/ethyl acetate/acetone (8/6/1→8/6/2) afforded **75** (0.158 g; 0.10 mmol; 64%): $[\alpha]_D$ 57 (c 0.40, CHCl3); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.22-8.19 (m, 2H, Ar), 7.57-7.53 (m, 1H, Ar), 7.51-7.45 (m, 4H, Ar), 7.35-7.30 (m, 5H, Ar), 7.28-7.15 (m, 18H, Ar), 5.77 (ddd, 1H, H-4$_{sph}$, J = 8.0 Hz, J = 10.4 Hz, J = 17.4 Hz), 5.69 (ddd, 1H, H-8", $J_{8",9a"}$ = 2.5 Hz, $J_{8",9b"}$ = 6.1 Hz, $J_{8",7"}$ = 10.0 Hz), 5.39 (s, 1H, C*H*Ph), 5.35-5.32 (m, 1H, H-5a$_{sph}$), 5.29-5.23 (m, 3H, H-1', H-2', H-5b$_{sph}$), 5.22 (dd, 1H, H-7", $J_{7",6"}$ = 2.3 Hz), 5.12 (d, 1H, CH_2Ph, J = 11.7 Hz), 5.00 (d, 1H, N*H*, $J_{NH,5"}$ = 10.1 Hz), 4.89 (d, 1H, CH_2Ph, J = 11.7 Hz), 4.79 (ddd, 1H, H-4", $J_{4",5"}$ = 11.4 Hz, $J_{4",3"ax}$ = 12.8 Hz, $J_{4",3"eq}$ = 4.6 Hz), 4.76 (d, 1H, CH_2Ph, J = 11.3 Hz), 4.63 (d, 1H, CH_2Ph, J = 11.3 Hz), 4.55 (d, 1H, CH_2Ph, J = 11.9 Hz), 4.35 (dd, 1H, H-9a", $J_{9a",9b"}$ = 12.5 Hz), 4.31 (d, 1H, CH_2Ph, J = 11.9 Hz), 4.28 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 4.18 (d, 1H, CH_2Ph), 4.15 (d, 1H, CH_2Ph), 4.05 (dd, 1H, H-9b"), 4.01 (dd, 1H, H-6a', $J_{6a',6b'}$ = 12.5 Hz, $J_{6a',5'}$ ~ 0.5 Hz), 3.94-3.87 (m, 4H, H-4, H-1a$_{sph}$, H-5", H-3$_{sph}$), 3.84 (dd, 1H, H-3', $J_{3',2'}$ = 10.7 Hz, $J_{3',4'}$ = 3.2 Hz), 3.78-3.69 (m, 3H, H-6b', H-2$_{sph}$, H-6"), 3.74 (s, 3H, CO_2CH_3), 3.66 (dd, 1H, H-6a, $J_{6a,6b}$ = 10.7 Hz), 3.64 (dd, 1H, H-3, $J_{3,2}$ = $J_{3,4}$ = 8.8 Hz), 3.61 (d, 1H, H-4'), 3.57-3.53 (m, 2H, H-5', H-1b$_{sph}$), 3.43 (dd, 1H, H-6b, $J_{6b,5}$ = 6.8 Hz), 3.40-3.34 (m, 2H,

H-2, H-5), 2.56 (dd, 1H, H-3"$_{eq}$, $J_{3eq,3ax}$ = 12.8 Hz), 2.18 (s, 3H, C(O)CH_3), 2.04 (s, 3H, C(O)CH_3), 1.96 (s, 3H, C(O)CH_3), 1.87 (dd, 1H, H-3"$_{ax}$), 1.84 (s, 3H, C(O)CH_3), 1.73 (s, 3H, C(O)CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8, 170.7, 170.3, 170.1, 170.0, 169.7, 165.6, 139.3, 138.7, 138.4, 137.9, 137.8, 134.1, 133.1, 130.27, 130.25, 128.7, 128.5, 128.3, 128.2, 128.1, 128.00, 127.94, 127.87, 127.6 (2C), 127.4, 127.18, 127.17, 127.16, 126.7, 126.2, 120.7, 103.1, 101.9, 101.2, 83.2, 82.0, 81.2, 79.9, 77.7, 75.7, 75.0, 74.68, 74.66, 73.6, 72.8, 70.4, 69.8, 69.6, 69.4, 68.7, 68.3, 68.1, 67.1, 67.0, 64.2, 62.6, 53.0, 49.2, 46.0, 37.5, 23.2, 21.5, 20.84, 20.77, 20.68; ESI HRMS calc'd for $C_{79}H_{88}N_4O_{24}S$ Na: 1531.5406. Found: 1531.5407. Anal. calc'd for $C_{79}H_{88}N_4O_{24}S$: C 62.85, H 5.88, N 3.71. Found: C 62.61, H 6.02, N 3.55.

_S_-(Methyl 5-acetamido-4,7,8,9-tetra-_O_-acetyl-3,5-dideoxy-D-_glycero_-α-D-_galacto_-non-2-ulopyranosylonate)-(2→3)-(2-_O_-benzoyl-4,6-_O_-benzylidene-3-thio-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-_O_-benzyl-β-D-glucopyranosyl-(1→1)-(2_S_,3_R_)-2-(_N_-carboxybenzyl-6-aminohexan-amido)-3-_O_-benzyl-pent-4-ene-1,3-diol (76)

The azide **75** (0.150 g; 0.10 mmol) was dissolved in pyridine and water (9:1; 10 mL) then triphenylphosphine (0.052 g; 0.20 mmol; 2 eq.) was added and the mixture was heated at 50 °C for 4 hours before being allowed to cool to room temperature. 6-(Benzyloxycarbonylamino)-hexanoic acid succinimidyl ester (0.180 g; 0.50 mmol; 5 eq.) was added and the solution was heated at 50 °C overnight and then concentrated. Chromatography of the residue on silica gel using hexanes-ethyl acetate-acetone (4:3:2→3:3:2) as eluent afforded **76** (0.155 g; 0.09 mmol; 90%): $[\alpha]_D$ 53 (*c* 0.41, CHCl3); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.21-8.18 (m, 2H, Ar), 7.58-7.54 (m, 1H, Ar), 7.51-7.45 (m, 4H, Ar), 7.36-7.12 (m, 28H, Ar), 5.80 (d, 1H, NH_{sph}, $J_{NH,H2sph}$ = 8.9 Hz), 5.70-5.62 (m, 2H, H-8", H-4$_{sph}$), 5.41 (s, 1H, C*H*Ph), 5.29-5.24 (m, 2H, H-1', H-2'), 5.23

(dd, 1H, H-7", $J_{7",8"}$ = 2.3 Hz, $J_{7",6"}$ = 10.1 Hz), 5.19 (dd, 1H, H-5a$_{sph}$, J = 10.4 Hz, J = 1.5 Hz), 5.16 (d, 1H, CH_2Ph, J = 11.4 Hz), 5.12-5.06 (m, 2H, CH_2Ph, H-5b$_{sph}$), 5.01 (d, 1H, N*H*Ac, $J_{NH,H5"}$ = 10.0 Hz), 4.85 (d, 1H, CH_2Ph, J = 11.4 Hz), 4.82-4.75 (m, 2H, H-4", N*H*Z), 4.68 (d, 1H, CH_2Ph), 4.63 (d, 1H, CH_2Ph), 4.54 (d, 1H, CH_2Ph, J = 11.9 Hz), 4.34 (dd, 1H, H-9a", $J_{9a",8"}$ = 2. 6 Hz, $J_{9a",9b"}$ = 12.4 Hz), 4.30 (d, 1H, CH_2Ph, J = 11.9 Hz), 4.24 (bs, 2H, CH_2Ph), 4.21 (d, 1H, H-1, $J_{1,2}$ = 7.9 Hz), 4.17 (dd, 1H, H-1a$_{sph}$, $J_{1a,2}$ = 4.6 Hz, $J_{1a,1b}$ = 10.9 Hz), 4.11-4.06 (m, 2H, H-2$_{sph}$, H-6a'), 4.05 (dd, 1H, H-9b", $J_{9",8"}$ = 6.6 Hz), 4.00 (dd, 1H, H-4, $J_{4,3}$ = 8.8 Hz, $J_{4,5}$ = 9.6 Hz), 3.92-3.84 (m, 2H, H-5", H-3'), 3.83-3.78 (m, 2H, H-6b', H-3$_{sph}$), 3.75-3.72 (m, 1H, H-6"), 3.74 (s, 3H, CO_2CH_3), 3.67 (dd, 1H, H-6a, $J_{6a,5}$ = 1.6 Hz, $J_{6a,6b}$ = 10.9 Hz), 3.64 (bd, 1H, H-4'), 3.63 (dd, 1H, H-3, $J_{3,2}$ = 9.0 Hz), 3.59 (bs, 1H, H-5'), 3.56 (dd, 1H, H-1b$_{sph}$, $J_{1b,2}$ = 6.3 Hz), 3.52 (dd, 1H, H-6b, $J_{6b,5}$ = 6.3 Hz), 3.34 (dd, 1H, H-2), 3.30 (m, 1H, H-5), 3.10 (m, 2H, CH_2NHZ), 2.57 (dd, 1H, H-3"$_{eq}$, $J_{3"eq,4"}$ = 4.6 Hz, $J_{3"eq,3"ax}$ = 12.8 Hz), 2.16, 2.05, 1.97, (3 x s, 3 x 3H, C(O)CH_3), 1.88 (dd, 1H, H-3"$_{ax}$, $J_{3"ax,4"}$ = 12.0 Hz), 1.85-1.68 (m, 2H, CH_2), 1.84, 1.73, (2 x s, 3 x 3H, C(O)CH_3), 1.42-1.34 (m, 4H, 2 x CH_2), 1.16-1.08 (m, 2H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.3, 170.8, 170.7, 170.2, 170.1, 170.0, 169.7, 165.6, 156.3, 139.1, 138.5, 138.3, 138.2, 137.7, 136.7, 135.7, 133.1, 130.23, 130.21, 128.7, 128.489, 128.486, 128.478, 128.31, 128.29, 128.14, 128.13, 128.02, 128.00, 127.8, 127.7, 127.57, 127.53, 127.50, 127.3, 127.1, 126.9, 126.2, 119.3, 103.8, 101.5, 101.2, 83.4, 81.9, 81.2, 79.4, 75.6, 75.2, 74.8, 74.5, 73.6, 72.7, 70.5, 69.8, 69.5, 69.4, 68.8, 68.5, 68.1, 67.1, 66.9, 66.5, 62.5, 53.0, 52.2, 49.2, 45.9, 40.8, 37.5, 36.0, 29.7, 29.6, 26.2, 24.9, 23.2, 21.5, 20.9, 20.8, 20.7; ESI HRMS calc'd for $C_{93}H_{107}N_3O_{29}SNa$: 1752.6714. Found: 1752.6710. Anal. for $C_{93}H_{107}N_3O_{29}S$: C 64.53, H 6.23, N 2.43. Found: C 64.54, H 6.39, N 2.25.

5-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)-(4,6-*O*-benzylidene-3-thio-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-(*N*-carboxybenzyl-6-aminohexan-amido)-3-*O*-benzyl-pent-4-ene-1,3-diol (77)

The trisaccharide **76** (0.140 g; 0.08 mmol) was combined with methanol (7.0 mL) and a small piece of sodium was added. After evolution of gas had ceased, the mixture was heated at 55 °C for 7 hours and then cooled to room temperature. Water (0.350 mL) was added and the solution was heated at 40 °C overnight. After acidification with acetic acid, the reaction mixture was concentrated and the residue chromatographed on silica gel using a gradient of methanol and dichloromethane (0:100→25:75). The residue was dissolved in 50% methanol and loaded on a SepPak cartridge (5 g), then eluted with 80-90% methanol to afford **77** (0.108 g; 0.08 mmol; 94%): $[\alpha]_D$ 10 (c 0.34, CHCl3); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.43-7.38 (m, 4H, Ar), 7.36-7.34 (m, 2H, Ar), 7.33-7.28 (m, 10H, Ar), 7.28-7.20 (m, 11H, Ar), 7.10-7.04 (m, 3H, Ar), 5.74 (ddd, 1H, H-4$_{sph}$), 5.50 (s, 1H, C*H*Ph), 5.30-5.24 (m, 2H, H-5a$_{sph}$, H-5b$_{sph}$), 5.16 (d, 1H, CH_2Ph, J = 10.7 Hz), 5.03 (bs, 2H, CH_2Ph), 4.78 (d, 1H, CH_2Ph, J = 11.4 Hz), 4.63 (bd, 3H, 3 x CH_2Ph), 4.58-4.54 (m, 3H, CH_2Ph, CH_2Ph, H-1'), 4.43 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 4.38 (d, 1H, CH_2Ph, $J_{1,2}$ = 11.8 Hz), 4.17 (ddd, 1H, H-2$_{sph}$, $J_{1a,2}$ = 6.4 Hz, J = 6.6 Hz, J = 3.8 Hz), 4.12 (dd, 1H, H-1a$_{sph}$, $J_{1a,1b}$ = 10.1 Hz), 4.09 (dd, 1H, H-4', $J_{4,3}$ = 3.5 Hz, $J_{4,5}$ = 0.6 Hz), 4.06 (dd, 1H, H-6a', $J_{6a',5'}$ = 1.4 Hz, $J_{6a',6b'}$ = 12.4 Hz), 4.04-3.99 (m, 3H, H-8", H-4, H-6a), 3.95 (dd, 1H, H-6b, $J_{6a,5}$ = 1.6 Hz, $J_{6a,6b}$ = 11.0 Hz), 3.93-3.87 (m, 2H, H-3$_{sph}$, H-6b'), 3.82 (dd, 1H, H-9a", $J_{9a",8"}$ = 2.8 Hz, $J_{9a",9b"}$ = 11.4 Hz), 3.77-3.73 (m, 3H, H-1b$_{sph}$, H-5", H-4"), 3.69 (dd, 1H, H-9b", $J_{9b",8"}$ = 4.4 Hz), 3.65 (dd, 1H, H-3', $J_{3',4'}$ = 3.4 Hz, $J_{3',2'}$ = 11.0 Hz), 3.62 (dd, 1H, H-3, $J_{3,2}$ = $J_{3,4}$ = 9.1 Hz), 3.59 (dd, 1H, H-7", J = 1.8 Hz, J = 9.2 Hz), 3.56-3.52 (m,

2H, H-6", H-5), 3.47 (dd, 1H, H-2', $J_{2',1'}$ = 7.6 Hz, $J_{2',3'}$ = 11.0 Hz), 3.29 (m, 1H, H-2), 3.17 (bs, 1H, H-5'), 3.01 (m, 2H, CH_2NHZ), 2.94 (m, 1H, H-3"$_{eq.}$), 2.00 (s, 3H, NC(O)CH_3), 2.00 (m, 2H, CH_2), 1.70 (m, 1H, H-3"$_{ax}$), 1.47 (m, 2H, CH_2), 1.39 (m, 2H, CH_2), 1.21 (m, 2H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.6, 175.7, 175.4, 175.0, 140.2, 140.1, 140.0, 139.9, 139.8, 139.6, 136.9, 129.8, 129.6, 129.45, 129.39, 129.34, 129.28, 129.09, 129.03, 128.99, 128.98, 128.93, 128.85, 128.81, 128.7, 128.6, 128.5, 128.3, 127.4, 120.1, 105.3, 104.7, 102.2, 85.8, 84.6, 83.0, 80.8, 78.1, 77.6, 76.9, 76.0, 75.9, 74.2, 72.5, 71.8, 70.8, 70.4, 70.0, 69.81, 69.78, 69.6, 69.1, 67.3, 64.4, 54.0, 53.7, 50.9, 49.3, 42.9, 41.7, 37.1, 30.6, 27.3, 26.6, 22.6; ESI HRMS calc'd for $C_{77}H_{93}N_3O_{22}SNa$: 1466.5868. Found: 1466.5869.

5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid-(2→8)-[*S*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)]-(2→3)-(3-thio-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-(6-aminohexan-amido)-pent-4-ene-1,3-diol (78)

GM_3 analogue **52** (2.09 mg; 2.4 μmol) and crude CMP-Neu5Ac (21 mg; 7.3 μmol; 3.0 eq.) were combined as solids. To this mixture was added successively freshly prepared α(2-3/8) sialyltransferase[212] in 20 mM Tris buffer, pH 8.3 (2.0 mL; 1.33 U/mL), $MnCl_2$ (20 μL of a 0.5M solution), and alkaline phosphatase (2 μL; 2U). The mixture was tumbled overnight at room temperature before centrifugation (30 minutes at 14,000 rpm). The supernatant was loaded directly onto a Biogel P4 column (2x50 cm) and eluted with 5% methanol at a flow rate of 0.4 mLmin^{-1}. Fractions containing the tetrasaccharide were purified further by HPLC (Hamilton PRP-1 semipreparative column, water-methanol gradient) to afford **78** (1.1 mg; 1.0 μmol; 39%): ^{1}H NMR (500 MHz, D_2O) δ [note: α(2→3) and α(2→8) linked Neu5Ac residues could not be unambiguously

identified, but individual spin systems are denoted by $H_{\#}$ and H^{*}) 5.86 (ddd, 1H, H-4_{sph}, J = 6.9 Hz, J = 10.5 Hz, J = 17.2 Hz), 5.33 (ddd, 1H, H-$5a_{sph}$, J = 1.2 Hz, J = 1.2 Hz, J = 17.2 Hz), 5.28 (ddd, 1H, H-$5b_{sph}$), 4.54 (d, 1H, H-1', $J_{1',2'}$ = 7.0 Hz), 4.48 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 4.25 (dd, 1H, H-9a*, $J_{9a^{*},8^{*}}$ = 4.1 Hz, $J_{9a^{*},9b^{*}}$ = 12.4 Hz), 4.24-4.21 (m, 1H, H-3_{sph}), 4.17-4.10 (m, 2H, H-8*, H-2_{sph}), 4.07-4.01 (m, 2H, H-$1a_{sph}$, H-6a), 3.95-3.89 (m, 1H, H-$8_{\#}$), 3.89-3.78 (m, 8H, H-$9a_{\#}$, H-4', H-6b, H-5*, H-7*, H-$1b_{sph}$, H-$5_{\#}$, H-5'), 3.74-3.53 (m, 12H, H-6a', H-6b', H-4, H-4*, H-9b*, H-3, H-$9b_{\#}$, H-5, H-$6_{\#}$, H-6*, H-$7_{\#}$, H-$4_{\#}$), 3.42 (dd, 1H, H-3', $J_{3',4'}$ = 2.3 Hz, $J_{3',2'}$ = 11.5 Hz), 3.38 (dd, 1H, H-2'), 3.33 (dd, 1H, H-2, $J_{2,3}$ = 8.9 Hz), 3.02-2.97 (m, 2H, CH_2NH_2), 2.78 (dd, 1H, H-3eq*, $J_{3eq^{*},4^{*}}$ = 4.7 Hz, $J_{3eq^{*},3ax^{*}}$ = 12.3 Hz), 2.75 (dd, 1H, H-$3eq_{\#}$, $J_{3eq\#,4\#}$ = 4.3 Hz, $J_{3eq\#,3ax\#}$ = 12.3 Hz), 2.32-2.27 (m, 2H, C(O)CH_2), 2.07 (s, 3H, NHC(O)CH_3), 2.04 (s, 3H, NHC(O)CH_3), 1.77 (dd, 1H, H-$3ax_{\#}$, $J_{3ax\#,4\#}$ = 12.0 Hz), 1.73 77 (dd, 1H, H-$3ax^{*}$, $J_{3ax^{*},4^{*}}$ = 12.1 Hz), 1.70-1.59 (m, 4H), 1.43-1.36 (m, 2H); ESI HRMS calc'd for $C_{45}H_{75}N_4O_{28}SNa_3$: 1220.3971. Found: 610.1989 $[M + 2Na]^{2+}$.

2-(Trimethylsilyl)ethyl S-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonate)-(2→3)-(4,6-di-*O*-acetyl-2-*O*-benzoyl-3-thio-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (79)

The diol **48** (0.101 g; 75 μmol) was dissolved in pyridine/acetic anhydride (1:1) and stirred overnight at ambient temperature. After concentration of the solution, chromatography on silica gel with 1.5% methanol in dichloromethane as eluent afforded the title compound **79** (0.102 g; 71 μmol; 95%): $[\alpha]_D$ 55 (c 1.2, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11 (m, 2H, Ar), 8.01-7.96 (m, 4H, Ar), 7.90 (m, 2H, Ar), 7.55 (m, 1H, Ar), 7.48-7.31 (m, 11H, Ar), 5.61 (dd, 1H, H-3, $J_{3,2}$ = $J_{3,4}$ = 9.5 Hz), 5.56 (ddd, 1H, H-8", $J_{8",7"}$ = 10.3 Hz, $J_{8",9a"}$ = $J_{8",9b"}$ = 3.0 Hz), 5.37 (dd, 1H, H-2, $J_{2,1}$ = 7.9 Hz), 5.23 (dd, 1H,

H-7", $J_{7",6"}$ = 2.4 Hz), 5.10 (d, 1H, H-1', $J_{1',2'}$ = 7.6 Hz), 4.99-4.93 (m, 2H, H-2', N*H*), 4.75 (ddd, 1H, H-4", $J_{4",3e"}$ = 4.5 Hz, $J_{4",3a"}$ = 11.7 Hz, $J_{4",5"}$ = 10.6 Hz), 4.68 (dd, 1H, H-4', $J_{4',3'}$ = 3.0 Hz, $J_{4',5'}$ = 0.7 Hz), 4.65 (dd, 1H, H-6a, $J_{6a,5}$ = 1.9 Hz, $J_{6a,6b}$ = 12.0 Hz), 4.61 (d, 1H, H-1), 4.38 (m, 2H, H-6b, H-9a"), 4.27 (dd, 1H, H-9b", $J_{9b",9a"}$ = 13.1 Hz), 4.22 (dd, 1H, H-4, $J_{4,5}$ = 9.5 Hz), 3.91-3.82 (m, 2H, H-a, H-5), 3.75 (s, 3H, OCH_3), 3.74-3.66 (m, 3H, H-5, H-3', H-5"), 3.58 (dd, 1H, H-6", $J_{6",5"}$ = 10.8 Hz), 3.47 (ddd, 1H, H-a'), 3.41 (dd, 1H, H-6a', $J_{6a',5'}$ = 6.8 Hz, $J_{6a',6b'}$ = 11.4 Hz), (dd, 1H, H-6a', $J_{6b',5'}$ = 6.6 Hz), 2.48 (dd, 1H, H-3e, $J_{3e",3a"}$ = 12.8 Hz), 2.08, 2.01, 1.91, 1.84, 1.82, 1.78 (6 x s, 18H, $C(O)CH_3$), 1.72 (dd, 1H, H-3a"), 1.61 (s, 3H, $C(O)CH_3$), 0.79 (m, 2H, H-b, H-b'), -0.18 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.71, 170.66, 170.2, 170.1, 170.0, 169.9, 169.8, 168.8, 165.8, 165.23, 165.18, 165.12, 133.0, 132.9, 132.79, 132.78, 130.3, 130.1, 130.0, 129.8, 129.66, 129.59, 129.56, 129.0, 128.4, 128.23, 128.20, 128.0, 100.8, 100.4, 81.2, 74.8, 73.3, 73.2, 73.0, 72.1, 71.9, 69.3, 69.2, 68.5, 67.4, 67.1, 66.1, 62.8, 61.8, 61.7, 53.0, 49.1, 45.0, 36.7, 23.1, 20.9 (2C), 20.68, 20.66, 20.5, 20.3, 17.8, -1.6; ESI MS calc'd for $C_{69}H_{81}NO_{28}SSiNa$: 1454.4. Found: 1454.4. Anal. calc'd for $C_{69}H_{81}NO_{28}SSi$: C 57.85, H 5.70, N 0.98. Found: C 57.71, H 6.04, N 0.91.

_S_-(Methyl 5-acetamido-4,7,8,9-tetra-_O_-acetyl-3,5-dideoxy-D-_glycero_-α-D-_galacto_-non-2-ulopyranosylonate)-(2→3)-(4,6-di-_O_-acetyl-2-_O_-benzoyl-3-thio-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-_O_-benzoyl-α/β-D-glucopyranose (80)

The trisaccharide **79** (0.092 g; 64 μmol) was dissolved in a mixture of anhydrous toluene (2.0 mL) and trifluoroacetic acid (3.0 mL) with stirring under argon. After two hours, the solution was diluted with toluene (5.0 mL) and concentrated at room temperature to a volume of approximately 1 mL. The process was repeated three times, and then evaporated to dryness. Chromatography of the residue on silica gel with 2.5% methanol

in dichloromethane as eluent afforded the hemiacetal **80** as a mixture of anomers (82 mg; 62 μmol; 96%): ESI MS calc'd for $C_{64}H_{69}NO_{28}SNa$: 1354.4. Found: 1354.4. Anal. calc'd for $C_{64}H_{69}NO_{28}S$: C 57.70, H 5.22. Found: C 57.58, H 5.52.

_S_-(Methyl 5-acetamido-4,7,8,9-tetra-_O_-acetyl-3,5-dideoxy-D-_glycero_-α-D-_galacto_-non-2-ulopyranosylonate)-(2→3)-(4,6-di-_O_-acetyl-2-_O_-benzoyl-3-thio-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-_O_-benzoyl-β-D-glucopyranosyl)-(1→1)-(2_S_, 3_R_)-2-azido-3-_O_-benzoyl-4-pentene-1,3-diol (81)

The hemiacetal **80** (0.088 g; 66 μmol) was concentrated from dry toluene (10 mL) and dissolved in dichloromethane (4.0 mL) containing trichloroacetonitrile (0.132 mL; 1.32 mmol; 20 eq.). 1,8-Diazabicyclo-[5.4.0]-undec-7-ene (5.0 μL; 0.5 eq.) was added, and the reaction mixture was stirred under argon for 90 minutes, then concentrated to approximately one quarter of the initial volume (bath temperature < room temp). The remaining solution was passed through a short plug (2cm) of silica gel using toluene/acetone (5:2) as eluent. The residue obtained after concentration (ESI MS calc'd for $C_{66}H_{69}Cl_3N_2O_{28}SNa$: 1499.3. Found: 1499.3) was combined with (2_S_, 3_R_)-2-azido-3-_O_-benzoyl-pent-4-ene-1,3-diol (0.020 g; 86 μmol; 1.3 eq.), and the mixture was taken up in toluene and concentrated before Drierite (~ 0.1 g) was added, followed by dichloromethane, and the mixture was stirred under an argon atmosphere for 30 minutes, then cooled to –15 °C. After this time boron trifluoride-diethyl etherate (25 μL; 0.2 mmol; 3 eq.) was added, and stirring was continued for 24 hours. The mixture was neutralized with pyridine (0.1 mL) diluted with dichloromethane (25 mL), filtered, washed with water, and the organic layers dried over sodium sulfate then filtered and concentrated. The residue was chromatographed on silica gel employing toluene/acetone (5:2) as eluent to afford **81** (0.082 g; 53 μmol; 80%): $[\alpha]_D$ 31 (c 1.0, $CHCl_3$); ^1H NMR

(600 MHz, $CDCl_3$) δ 8.12-8.08 (m, 2H, Ar), 7.99-7.89 (m, 8H, Ar), 7.55-7.30 (m, 15H, Ar), 5.74 (ddd, 1H, H-4_{sph}, $J_{4,5b}$ = 10.5 Hz, $J_{4,5a}$ = 17.3 Hz, $J_{4,3}$ = 7.0 Hz, 5.63 (dd, 1H, H-3, $J_{3,2} = J_{3,4}$ = 9.4 Hz), 5.57 (ddd, 1H, H-8", $J_{8",7"}$ = 2.7 Hz, $J_{8",9a"}$/ $J_{8",9b"}$ = 9.8 Hz, 3.2 Hz), 5.50 (dd, 1H, H-$3_{N\text{-acyl}}$, $J_{3,4}$ = 3.7 Hz), 5.41 (dd, 1H, H-2, $J_{2,1}$ = 7.7 Hz, $J_{2,3}$ = 9.7 Hz), 5.23 (dd, 1H, H-7", $J_{7",6"}$ = 10.4 Hz), 5.19 (ddd, 1H, H-$5a_{sph}$), 5.13 (ddd, 1H, H-$5b_{Sph}$), 5.11 (d, 1H, H-1', $J_{1',2'}$ = 7.6 Hz), 5.0-4.94 (m, 2H, H-2', N*H*), 4.75 (ddd, 1H, H-4", $J_{4",3e"}$ = 4.6 Hz, $J_{4",3a"}$ = 11.7 Hz, $J_{4",5"}$ = 10.4 Hz), 4.70-4.62 (m, 3H, H-1, H-6a, H-4'), 4.40-4.35 (m, 2H, H-6b, H-9a"), 4.28-4.22 (m, 2H, H-4, H-9b"), 3.93-3.80 (m, 3H, H-5', H-2_{Sph}, H-$1a_{Sph}$), 3.76 (s, 3H, OCH_3), 3.74-3.66 (m, 3H, H-5, H-3', H-5"), 3.57 (m, 2H, H-$1b_{Sph}$), H-6"), 3.44 (dd, 1H, H-6a', $J_{6a',5'}$ = 6.7 Hz, $J_{6a',6b'}$ = 11.3 Hz), 3.33 (dd, 1H, H-6a', $J_{6a',5'}$ = 6.6 Hz), 2.48 (dd, 1H, H-3e", $J_{3e",3a"}$ = 12.7 Hz), 2.07, 2.02, 1.91, 1.84, 1.83, 1.77 (6 x s, 18H, $C(O)CH_3$), 1.72 (dd, 1H, H-3a"), 1.62 (s, 3H, $C(O)CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.69, 170.66, 170.13, 170.08, 170.02, 169.91, 161.8, 168.8, 165.66, 165.18, 165.13, 165.06, 164.8, 133.12, 133.11, 133.09, 132.88, 132.82, 131.1, 130.1, 129.99, 129.95, 129.80, 129.78, 129.68, 129.63, 129.60, 129.52, 129.3, 128.42, 128.34, 128.28, 128.26, 128.1, 120.4, 100.82, 100.78, 81.2, 74.6, 74.5, 73.4, 73.3, 72.8, 72.1, 71.5, 69.4, 69.1, 68.5, 67.9, 67.1, 66.1, 63.1, 62.5, 61.9, 61.7, 53.0, 49.0, 44.9, 36.7, 23.1, 20.92, 20.85, 20.7 (2C), 20.5, 20.3; ESI MS calc'd for $C_{76}H_{80}N_4O_{30}SNa$: 1583.4. Found: 1583.4.

_S_-(Methyl 5-acetamido-4,7,8,9-tetra-_O_-acetyl-3,5-dideoxy-D-_glycero_-α-D-_galacto_-non-2-ulopyranosylonate)-(2→3)-(4,6-di-_O_-acetyl-2-_O_-benzoyl-3-thio-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-_O_-benzoyl-β-D-glucopyranosyl)-(1→1)-(2_S_, 3_R_)-3-_O_-benzoyl-2-octadecylamido-pent-4-ene-1,3-diol (82)

Azide **81** (0.028 g; 18 μmol) was dissolved in pyridine/water (9:1) with stirring. Triphenylphosphine (0.007 g; 27 μmol; 1.5 eq.) was added, and the mixture was stirred at 40 °C for 7 hours. *N*-(Octadecanoyloxy)-succinimide (0.020 g; 54 μmol; 3.0 eq.) was added, and stirring was continued overnight. Concentration of the solution, followed by chromatography on silica gel using a linear gradient of methanol in dichloromethane (0→2.25%) as eluent afforded a colourless solid (0.023 g; 13 μmol; 71%): $[\alpha]_D$ 47 (c 0.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10-7.86 (m, 10H, Ar), 7.56-7.22 (m, 15H, Ar), 5.79 (ddd, 1H, H-4$_{sph}$, $J_{4,5b}$ = 10.8 Hz, $J_{4,5a}$ = 17.2 Hz, $J_{4,3}$ = 6.3 Hz, 5.66-5.59 (m, 2H, *H*NC(O)$C_{17}H_{35}$, H-3), 5.56 (m, 1H, H-8"), 5.48 (dd, 1H, H-3$_{sph}$, $J_{3,4} = J_{3,2}$ = 6.3 Hz), 5.36 (dd, 1H, H-2, $J_{2,1}$ = 7.9 Hz, $J_{2,3}$ = 10.0 Hz), 5.26-5.20 (m, 2H, H-5a$_{sph}$, H-7"), 5.13 (ddd, 1H, H-5b$_{sph}$), 5.08 (d, 1H, H-1', $J_{1',2'}$ = 7.5 Hz), 5.0-4.9 (m, 2H, H-2', N*H*), 4.75 (ddd, 1H, H-4", $J_{4",3e"}$ = 4.7 Hz), 4.69 (dd, 1H, H-4', $J_{4',3'}$ = 3.2 Hz, $J_{4',5'}$ = < 1 Hz), 4.56 (d, 1H, H-1, $J_{1,2}$ = 7.7 Hz), 4.52 (dd, 1H, H-6a, $J_{6a,5}$ = 1.9 Hz, $J_{6a,6b}$ = 12.2 Hz), 4.44-4.34 (m, 2H, H-2$_{sph}$, H-9a"), 4.28-4.22 (m, 2H, H-6b, H-9b"), 4.20 (dd, 1H, H-4, $J_{4,3} = J_{4,5}$ = 9.6 Hz, 4.04 (dd, 1H, H-1a$_{sph}$, $J_{1a,1b}$ = 10.0 Hz, $J_{1a,2}$ = 3.3 Hz), 3.90 (ddd, 1H, H-5', $J_{5',6a'} = J_{5',6b'}$ = 6.5 Hz), 3.75 (s, 3H, OCH_3), 3.74-3.67 (m, 2H, H-3', H-5"), 3.64 (m, 1H, H-5), 3.59 (dd, 1H, H-6", *J* = 10.7 Hz, 2.4 Hz), 3.44 (dd, 1H, H-6a', $J_{6a',6b'}$ = 11.4 Hz), 3.33 (dd, 1H, H-6a'), 2.48 (dd, 1H, H-3e", $J_{3e",3a"}$ = 12.8 Hz), 2.08, 2.03, 1.91, 1.85, 1.81, 1.78 (6 x s, 18H, C(O)CH_3), 1.76-1.70 (m, 2H, H-3a", H-1a$_{N\text{-acyl}}$, H-1b$_{N\text{-acyl}}$), 1.62 (s, 3H, C(O)CH_3), 1.36-1.10 (m, 30H), 0.86 (t, 3H, $CH_{3\text{-N-acyl}}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.67, 170.71, 170.66, 170.11, 170.09, 170.03, 169.9, 169.8, 168.8, 165.6, 165.3, 165.11, 165.09, 164.08, 133.4, 133.13, 133.07, 132.9, 132.8, 130.1, 130.0, 129.98, 129.96, 129.85, , 129.78, 129.74, 129.6, 129.50, 129.49, 129.1, 128.47, 128.40, 128.24, 128.23,

128.1, 118.7, 114.7, 100.80, 100.73 , 81.2, 74.3, 74.2, 73.4, 73.2, 72.6, 72.10, 72.08, 69.3, 69.1, 68.5, 67.3, 67.1, 66.1, 62.5, 61.9, 61.7, 53.0, 50.2, 49.0, 44.9, 38.1, 36.7, 36.4, 31.9, 31.2, 29.67 (multiple C), 29.64, 29.62, 29.61, 29.5, 29.32, 29.28, 29.1, 25.4, 23.1, 22.7, 20.93, 20.87, 20.69, 20.68, 20.47, 20.32, 14.8; ESI MS calc'd for $C_{94}H_{116}N_2O_{31}SNa$: 1824.7. Found: 1824.7.

_S_-(Methyl 5-acetamido-4,7,8,9-tetra-_O_-acetyl-3,5-dideoxy-D-_glycero_-α-D-_galacto_-non-2-ulopyranosylonate)-(2→3)-(4,6-di-_O_-acetyl-2-_O_-benzoyl-3-thio-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-_O_-benzoyl-β-D-glucopyranosyl)-(1→1)-(2_S_, 3_R_)-3-_O_-benzoyl-2-(_S_-Acetyl-16-mercaptohexadecan-amido)-pent-4-ene-1,3-diol (83)

Azide **81** (0.038 g; 24 μmol) and triphenylphosphine (0.013 g; 49 μmol; 2.0 eq.) were dissolved in a mixture of pyridine and water (9:1; 4.0 mL). The solution was heated at 45°C for three hours, *N*-(*S*-Acetyl-16-mercaptohexadecanoyloxy)-succinimide[280] (0.052 g; 96 μmol; 4.0 eq.) was added, and stirring was continued at elevated temperature for a further five hours. The solution was then concentrated and the residue chromatographed on silica gel using methanol in dichloromethane (2.2%) as eluent, yielding the thioacetate **83** (0.034 g; 18 μmol; 74%): $[\alpha]_D$ 49 (c 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.08 (m, 2H, Ar), 7.97 (m, 2H, Ar), 7.94-7.86 (m, 6H, Ar), 7.56-7.28 (m, 15H, Ar), 5.79 (ddd, 1H, H-4$_{sph}$, $J_{4,5b}$ = 10.6 Hz, $J_{4,5a}$ = 16.9 Hz, $J_{4,3}$ = 6.2 Hz, 5.66-5.61 (m, 2H, *H*NC(O)$C_{15}H_{30}$SAc, H-3), 5.56 (ddd, 1H, H-8", J = 2.7 Hz, J = 3.0 Hz, J = 10.0 Hz), 5.49 (dd, 1H, H-3$_{sph}$, $J_{3,4} = J_{3,2}$ = 6.3 Hz), 5.36 (dd, 1H, H-2, $J_{2,1}$ = 7.8 Hz, $J_{2,3}$ = 9.9 Hz), 5.26-5.21 (m, 2H, H-5a$_{sph}$, H-7"), 5.13 (ddd, 1H, H-5b$_{sph}$, J = 1.2 Hz, J = 1.2 Hz, J = 10.6 Hz), 5.07 (d, 1H, H-1', $J_{1',2'}$ = 7.6 Hz), 4.94-4.91 (m, 2H, H-2', N*H*), 4.75 (ddd, 1H, H-4", $J_{4",3e"}$ = 4.7 Hz, J = 10.3 Hz, J = 11.6 Hz), 4.69 (dd, 1H, H-4', $J_{4',3'}$ = 3.0 Hz, $J_{4',5'}$ = < 1 Hz), 4.56 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 4.53 (dd, 1H, H-6a, $J_{6a,5}$ = 1.6 Hz, $J_{6a,6b}$ = 11.9 Hz),

4.41-4.36 (m, 2H, H-2_{sph}, H-9a"), 4.31-4.24 (m, 2H, H-6b, H-9b"), 4.19 (dd, 1H, H-4, $J_{4,3}$ = $J_{4,5}$ = 9.5 Hz), 4.04 (dd, 1H, H-$1a_{sph}$, $J_{1a,1b}$ = 10.0 Hz, $J_{1a,2}$ = 3.3 Hz), 3.90 (ddd, 1H, H-5', $J_{5',6a'}$ = $J_{5',6b'}$ = 6.5 Hz), 3.75 (s, 3H, OCH_3), 3.77-3.64 (m, 2H, H-3', H-5"), 3.65 (m, 1H, H-5), 3.58 (dd, 1H, H-6", J = 10.8 Hz, 2.5 Hz), 3.43 (dd, 1H, H-6a', $J_{6a',6b'}$ = 11.3 Hz), 3.36 (dd, 1H, H-6a'), 2.83 (dd, 2H, CH_2SAc, J = 7.4 Hz), 2.48 (dd, 1H, H-3e", $J_{3e",3a"}$ = 12.6 Hz), 2.29, 2.08, 2.03, 1.91, 1.86, 1.81, 1.78 (7 x s, 21H, C(O)CH_3), 1.76-1.69 (m, 3H, H-3a", CH_2), 1.62 (s, 3H, C(O)CH_3), 1.53 (m, 2H, CH_2, 1.36-1.02 (m, 24H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.0, 172.6, 170.72, 170.65, 170.10, 170.08, 170.03, 169.9, 169.8, 165.5, 165.3, 165.10, 165.08, 165.06, 133.4, 133.11, 133.07, 132.91, 132.90, 132.8, 130.1, 129.96, 129.93, 129.8, 129.7, 129.6, 129.49, 129.46, 129.1, 128.5, 128.44, 128.39, 128.38, 128.23, 128.22, 128.06, 118.7, 100.78, 100.70, 81.1, 74.3, 74.1, 73.3, 73.2, 72.5, 72.07, 72.04, 69.3, 69.1, 68.5, 67.30, 67.29, 67.1, 66.1, 62.5, 61.8, 61.6, 53.0, 50.1, 49.0, 44.9, 36.6, 36.3, 30.6, 29.61, 29.60, 29.5, 29.4 (multiple C), 29.3, 29.12, 29.08, 28.8, 25.4, 23.1, 20.92, 20.86, 20.69, 20.68, 20.46, 20.30; ESI MS calc'd for $C_{94}H_{114}N_2O_{32}SNa$: 1870.7. Found: 1870.7.

_S_-(5-acetamido-3,5-dideoxy-D-_glycero_-α-D-_galacto_-non-2-ulopyranosylonic acid)-(2→3)-(3-thio-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2_S_, 3_R_)-2-octadecylamido-4-pentene-1,3-diol (84)

Glycolipid **82** (0.015 g; 8.3 μmol) was combined with dry methanol (4.0 mL) and a small piece of sodium (~5mg) was added. The solution was stirred for 24 hours and then water (400 μL) was added. Stirring was continued for a further 24 hours, then the mixture was neutralized with acidic ion exchange resin, filtered, and concentrated. The residue was loaded onto a preconditioned SepPak cartridge (0.4 g) in 10% methanol, washed extensively with water and eluted with methanol. Concentration of the eluate gave a

residue which was further purified by HPLC (Kromasil C_{18} column, water methanol gradient) to afford compound **84** (6.4 mg; 76%): $[\alpha]_D$ 21 (c 0.2, MeOH); ^{1}H NMR (500 MHz, CD_3OD, δ) 5.86 (ddd, 1H, H-4$_{Sph}$, $J_{4,5a}$ = 17.2 $J_{4,5b}$ = 10.4 Hz $J_{4,3}$ = 6.7 Hz), 5.26 (ddd, 1H, H-5a$_{sph}$, J = 17.1 Hz, 1.3 Hz, 1.7 Hz), 5.13 (ddd, 1H, H-5b$_{sph}$, J = 10.4 Hz, 1.2 Hz, 1.9 Hz), 4.46 (d, 1H, H-1', $J_{1',2'}$ = 7.6 Hz), 4.29 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 4.19-4.12 (m, 2H, H-1a$_{sph}$, H-3$_{sph}$), 4.02-3.95 (m, 2H, H-2$_{sph}$, H-8"), 3.93 (d, 2H, H-6a, H-6b, J = 3.4 Hz), 3.87-3.84 (m, H-4', H-9a"), 3.73-3.66 (m, H, H-4", H-5", H-6a'), 3.67-3.49 (m, 8H, H-5', H-6b', H-4, H-1b$_{sph}$, H-9b", H-3, H-6", H-3'), 3.44-3.38 (m, 3H, H-5, H-7", H-2'), 3.28 (dd, 1H, H-2, $J_{2,3}$ = 9.3 Hz), 2.91 (dd, 1H, H-3$_{eq}$", $J_{3e,4}$ = 4.4 Hz, $J_{3e,3a}$ = 12.3 Hz), 2.18 (t, 2H, C(O)CH_2, J = 7.6 Hz), 1.99 (s, 3H, NHAc), 1.70 (dd, 1H, H-3$_{ax}$", $J_{3a,4}$ =10.8 Hz), 1.62-1.56 (m, 2H, C(O)CH$_2$CH_2), 1.36-1.24 (m, 28H, alkyl), 0.50 (t, 3H, CH_3); ^{13}C NMR (125 MHz, CD_3OD, δ) 176.2, 175.4, 175.2, 139.7, 116.9, 106.4, 104.5, 85.3, 80.7, 79.8, 77.2, 76.5, 76.2, 74.9, 73.2, 72.9, 70.8, 70.41, 70.39, 64.9, 62.9, 61.70, 49.6, 37.2, 33.1, 30.78 (multiple C), 30.75, 30.74, 30.6, 30.49, 30.46, 30.35, 27.1, 23.7, 22.5, 14.4; ESI HRMS calc'd for $C_{46}H_{82}N_2O_{20}SNa$: 1037.5079. Found: 1037.5078.

_S_-(5-Acetamido-3,5-dideoxy-D-_glycero_-α-D-_galacto_-non-2-ulopyranosylonic acid)-(2→3)-(3-thio-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl)-(1→1)-(2_S_, 3_R_)-2-(16-mercaptohexadecan-amido)-pent-4-ene-1,3-diol (85)

Glycolipid **83** (0.010 g; 5.4 μmol) was dissolved in dry degassed methanol (2.0 mL) and a small piece of sodium was added. A gentle stream of argon was bubbled through the solution, and stirring was continued for approximately 24 hours. Water (350 μL) was added, and the solution was heated at 40 °C for 48 hrs. The solution was then cooled and dithiothreitol (0.010 g) was added. After stirring overnight, the mixture was neutralized with acidic resin, filtered, and concentrated. The residue was redissolved in 10%

methanol and applied to a SepPak cartridge (0.4 g). Gradient elution (MeOH-H_2O) afforded **85** which was further purified by HPLC (Beckman column, water-methanol gradient) (0.0045 g; 4.4 μmol; 82%): $[\alpha]_D$ 18 (c 0.26, MeOH); ^{1}H NMR (600 MHz, MeOH, δ) 5.86 (ddd, 1H, H-4$_{Sph}$, $J_{4,5a}$ = 17.1 $J_{4,5b}$ = 10.4 Hz $J_{4,3}$ = 6.7 Hz), 5.26 (ddd, 1H, H-5a$_{sph}$, J = 17.1 Hz, 1.4 Hz, 1.8 Hz), 5.13 (ddd, 1H, H-5b$_{sph}$, J = 10.4 Hz, 1.1 Hz, 1.8 Hz), 4.46 (d, 1H, H-1', $J_{1',2'}$ = 7.5 Hz), 4.29 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 4.18-4.12 (m, 2H, H-1a$_{sph}$, H-3$_{sph}$), 4.02-3.96 (m, 2H, H-2$_{sph}$, H-8"), 3.92 (d, 2H, H-6a, H-6b, J = 3.3 Hz), 3.87-3.83 (m, H-4', H-9a"), 3.75-3.67 (m, H, H-4", H-5", H-6a'), 3.67-3.50 (m, 8H, H-5', H-6b', H-4, H-1b$_{sph}$, H-9b", H-3, H-6", H-3'), 3.44-3.37 (m, 3H, H-5, H-7", H-2'), 3.28 (dd, 1H, H-2, $J_{2,3}$ = 9.1 Hz), 2.90 (dd, 1H, H-3$_{eq}$", $J_{3e,4}$ = 4.2 Hz, $J_{3e,3a}$ = 12.4 Hz), 2.44 (t, 2H, CH_2SH, J = 7.5 Hz),), 2.18 (t, 2H, C(O)CH_2, J = 7.1 Hz), 1.99 (s, 3H, NHAc), 1.69 (dd, 1H, H-3$_{ax}$", $J_{3a,4}$ = 10.7 Hz), 1.61-1.54 (m, 4H, alkyl), 1.42-1.25 (m, 22H, alkyl); ESI HRMS calc'd for $C_{44}H_{77}N_2O_{20}S_2Na_2$: 1063.4303. Found: 1063.4301.

β-D-Galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*)-2-butanamido-pent-4-ene-1,3-diol (86)

(β-D-Galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*)-2-amino-pent-4-ene-1,3-diol[166] (0.020 g; 0.045 mmol) was dissolved in methanol (2.0 mL) and butyric anhydride (0.019 mL; 113 μmol; 2.5 eq.) was added. After stirring for 2.5 hours the solution was concentrated and redissolved in water (1.5 mL). The mixture was applied to a SepPak cartridge (0.4 g) and eluted with water to afford **86** (0.0200 g; 0.0390 mmol; 86%): $[\alpha]_D$ 9 (c 0.15, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 5.86 (ddd, 1H, H-4$_{sph}$, $J_{4,5a}$ = 17.2 Hz, $J_{4,5b}$ = 10.5 Hz, $J_{4,3}$ = 7.0 Hz), 5.33 (bd, 1H, H-5a$_{sph}$, $J_{5a,4}$ = 17.2 Hz), 5.27 (bd, 1H, H-5b$_{sph}$, $J_{4,5b}$ = 10.5 Hz), 4.48 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 4.45 (d, 1H, H-1', $J_{1',2'}$ =

7.9 Hz), 4.23 (dd, 1H, H-3_{sph}, $J_{2,3}$ = 7.1 Hz, $J_{2,1}$ = 7.1 Hz), 4.11 (ddd, 1H, H-2_{sph}, $J_{2,3}$ = 7.1 Hz, $J_{2,1a}$ = 6.7 Hz, $J_{2,1b}$ = 3.7 Hz), 4.06 (dd, 1H, H-$1a_{sph}$, $J_{1a,1b}$ = 10.5 Hz, $J_{1a,2}$ = 6.4 Hz), 3.98 (dd, 1H, H-6a, $J_{6a,6b}$ = 12.3 Hz, $J_{6a,5}$ = 2.1 Hz), 3.93 (d, 1H, H-4', $J_{4',3'}$ = 3.3 Hz), 3.83-3.71 (m, 5H, H-6b, H-$1b_{sph}$, H-6a', H-6b', H-5'), 3.68-3.62 (m, 3H, H-3', H-3, H-4), 3.62-3.57 (m, 1H, H-5), 3.54 (dd, 1H, H-2', $J_{2,3}$ = 9.9 Hz, $J_{2,1}$ = 7.9 Hz), 3.37-3.32 (m, 1H, H-2) 2.23 (t, 2H, J = 7.4 Hz, CH_2C(O)N), 1.62-1.58 (sextet, 2H, CH_2CH$_3$, J = 7.4 Hz), 0.90 (t, 3H, CH_3, J = 7.4 Hz); ^{13}C NMR (from HMQC/HMBC spectra, 150 MHz, D_2O) δ 178.2, 137.2, 119.0, 103.8, 103.2, 79.5, 79.4, 76.4, 75.4, 75.2, 73.5, 73.3, 72.8, 71.8, 69.6, 69.4, 62.0, 53.9, 38.8, 19.9, 13.7; ESI HRMS calc'd for $C_{21}H_{37}NO_{13}Na$: 534.2157. Found: 534.2151.

(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-(butanamido)-pent-4-ene-1,3-diol (87)

Lactoside **86** (0.020 g; 39 μmol) and crude CMP-Neu5Ac (~ 0.0470 mmol; ~1.2 eq.) were combined as solids. 0.5 M $MnCl_2$ (100 μL; 10 mM final conc.), 0.5 M $MgCl_2$ (100 μL; 10 mM final conc.), 0.4 M dithiothreitol (32 μL; 2.5 mM final conc.), α(2,3)-sialyltransferase[217] (4.0 mL; 0.5 U/mL) and alkaline phosphate (5 μL; 5U) were then added sequentially and the mixture was tumbled gently overnight. When starting material had been consumed as judged by TLC ($CHCl_3$-MeOH-H_2O-AcOH; 10:7:1:2), the mixture was centrifuged (30 min at 14,000 rpm) and the supernatant was applied to a SepPak (5 g) which was eluted with water (30 mL), followed by a gradient of water-methanol. The product obtained was further purified by HPLC (Kromasil C_{18}-silica semi-preparative column) using water and methanol as eluents to afford (**87**) (0.0249 g; 0.0310 mmol; 79%): $[\alpha]_D$ 2 (c 0.30, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 5.85 (dddd 1H,

H-4$_{sph}$, $J_{4,5}$ = 17.3 Hz, $J_{4,5}$ = 10.4 Hz, $J_{4,3}$ = 7.0 Hz, $J_{4,2}$ = 1.1 Hz), 5.32 (dd, 1H, H-5$_{sph}$, $J_{5,4}$ = 17.3 Hz, $J_{5,5}$ = 1.1 Hz), 5.27 (dd, 1H, H-5$_{sph}$, $J_{5,4}$ = 10.6 Hz, $J_{5,5}$ = 1.1 Hz), 4.52 (dd, 1H, H-1, $J_{1,2}$ = 7.8 Hz, $J_{1,3}$ = 0.9 Hz), 4.47 (dd, 1H, H-1, $J_{1,2}$ = 7.9 Hz, $J_{1,3}$ = 0.9 Hz), 4.22 (ddd, 1H, H-3$_{sph}$, $J_{3,4}$ = 7.1 Hz, $J_{3,2}$ = 7.1 Hz, $J_{3,1}$ = 0.9 Hz), 4.12-4.05 (m, 3H, H-3', H-2$_{sph}$, H-1a$_{sph}$), 3.99 (d, 1H, $J_{6a,6b}$ = 11.4 Hz, $J_{6a,5}$ = 1.8 Hz, H-6a), 3.95 (d, 1H, $J_{4,3}$ = 2.7 Hz, H-4'), 3.90-3.73 (m, 5H, H-8", H-5", H-9a", H-6b, H-1b$_{sph}$), 3.71-3.62 (m, 8H, H-4", H-6a', H-6b', H-5', H-3, H-4, H-9b", H-6"), 3.60-3.55 (m, 3H, H-5, H-7", H-2'), 3.35-3.32 (m, 1H, H-2), 2.75 (dd, 1H, $J_{3e",3a"}$ = 12.5 Hz, $J_{3e",4"}$ = 4.6 Hz, H-3"eq), 2.22 (t, 2H, J = 7.1 Hz, CH_2C(O)), 2.03 (s, 3H, C(O)CH_3), 1.79 (dd, 1H, $J_{3a",3e"}$ = 12.1 Hz, $J_{3a",4"}$ = 12.1 Hz, H-3"ax), 1.62-1.56 (m, 4H, CH_2CH$_2$C(O), CH_2CH$_2$CH$_2$C(O)), 0.89 (t, 3H, J = 9.3 Hz, CH_3); ^{13}C NMR (125 MHz, D_2O) δ 178.1, 175.9, 174.8, 137.1, 119.0, 103.6, 103.3, 100.7, 79.2, 76.4, 76.1, 75.6, 75.1, 73.8, 73.7, 72.74, 72.66, 70.3, 69.5, 69.2, 69.0, 68.4, 63.5, 61.9, 61.0, 53.9, 52.6, 40.7, 38.7, 22.9, 19.9, 13.7; ESI HRMS calc'd for $C_{32}H_{53}N_2O_{21}$: 801.3134. Found: 801.3146.

(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid-(2→3)-[2-acetamido-2-deoxy-(β-D-galactopyranosyl)-(1→4)]-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-(butanamido)-pent-4-ene-1,3-diol (88)

UDP-*N*-acetylglucosamine disodium salt (0.015 g; 0.0180 mmol; 1.2 eq.) was added to a Falcon tube containing (**87**) (0.012 g; 0.0150 mmol; 1.0 eq.). Freshly prepared *N*-acetylgalactosaminyl transferase[213] (2.0 mL; 0.8 U/mL) in 50 mM HEPES, pH 7.5 containing 10% glycerol (v/v) and UDP *N*-acetylglucosamine 4-epimerase[212] (2.0 mL; unknown activity) containing 0.2 M NaCl, 1.0 mM EDTA, 20mM HEPES, 5 mM β-mercaptoethanol (pH 7.0) were added, followed by 0.5M $MnCl_2$ (4.0 μL; 0.5 mM final

conc.) and alkaline phosphatase (8 μL; 8 U). The mixture was tumbled gently overnight and centrifuged for 20 min at 14000 rpm. The supernatant was applied to a SepPak cartridge (5 g), washed with water and eluted with a gradient of water-methanol. After concentration the residue was taken up in 5% methanol and purified on a Biogel P4 column (50 cm x 2 cm) eluted at 0.4 mL min^{-1} to yield **88** (0.0126 g; 0.0130 mmol; 83%): $[\alpha]_D$ 15 (c 0.73, H_2O). ^1H NMR (600 MHz, D_2O) δ 5.85 (ddd, 1H, H-4_{sph}, $J_{4,5a}$ = 17.3 Hz, $J_{4,5b}$ = 10.4 Hz, $J_{4,3}$ = 7.0 Hz), 5.32 (d, 1H, H-$5a_{sph}$, $J_{5a,4}$ = 17.2 Hz), 5.26 (d, 1H, H-$5b_{sph}$, $J_{5,4}$ = 10.6 Hz), 4.73 (m, 1H, H-1'''), 4.51 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1'), 4.47 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 4.22 (dd, 1H, H-3_{sph}, $J_{3,4}$ = 7.0 Hz, $J_{3,2}$ = 7.0 Hz), 4.14 (dd, 1H, H-3', $J_{3',2'}$ = 9.8 Hz, $J_{3',4'}$ = 3.0 Hz), 4.11-4.05 (m, 3H, H-4', H-2_{sph}, H-$1a_{sph}$), 3.99 (dd, 1H, H-6a, $J_{6a,6b}$ = 12.2 Hz, $J_{6a,5}$ = 1.9 Hz), 3.92-3.89 (m, 2H, H-4''', H-2''') 3.87-3.58 (m, 18H, H-9a'' H-5'', H-6b, H-$1b_{sph}$, H-4'', H-5', H-6a', H-6b', H-5''', H-6a''', H-6b''', H-8'', H-3''', H-3, H-9b'', H-4, H-7'', H-5), 3.48 (dd, 1H, H-6'', $J_{6,5}$ = 10.1 Hz, $J_{6,7}$ = 1.8 Hz), 3.37-3.31 (m, 2H, H-2', H-2), 2.65 (dd, 1H, H-3''eq, $J_{3e,3a}$ = 12.6 Hz, $J_{3e,4}$ = 4.5 Hz), 2.22 (t, J = 7.3 Hz, $CH_2C(O)$), 2.02, 2.01 (2 x s, 6H, $C(O)CH_3$), 1.91 (dd, 1H, H-3''ax, $J_{3a'',3e''}$ = 11.8 Hz, $J_{3a'',4''}$ = 11.8 Hz) 1.61-1.55 (m, 4H, $CH_2CH_2CH_2C(O)$, $CH_2CH_2C(O)$), 0.89 (t, 3H, CH_3); ^{13}C NMR (125 MHz, D_2O) δ 178.0 (2C), 175.9, 174.9, 137.1, 119.0, 103.6, 103.5, 103.2, 102.5, 79.5, 78.0, 75.60, 75.59, 75.2, 75.1, 74.9, 74.0, 73.6, 73.1, 72.7, 72.2, 70.9, 69.6, 69.5, 68.9, 68.7, 63.7, 62.0, 61.4, 61.0, 53.9, 53.2, 52.5, 38.7, 37.9, 23.5, 22.9, 19.9, 13.7. ESI HRMS calc'd for $C_{40}H_{66}N_3O_{26}$: 1004.3940. Found: 1004.3940.

(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid-(2→3)-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-azido-pent-4-ene-1,3-diol (89)

β-D-Galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-azido-pent-4-ene-1,3-diol[166] (0.0201 g; 43 μmol) was combined with CMP-sialic acid (0.110 g of crude; ~ 5.1 μmol; ~ 1.1 eq.). To this mixture was added successively α(2→3) sialyltransferase[217] in 50 mM HEPES (pH 7.5), containing 10% glycerol (v/v) (2.36 mL; 0.14 U/mL), 0.5 M $MnCl_2$ (50 μL; 10 mM final conc.); 0.5 M $MgCl_2$ (50 μL; 10 mM final conc.); 0.4 M dithiothreitol (19 μL; 3 mM final conc.) and alkaline phosphatase (5 μL; 5U). The mixture was tumbled overnight and then centrifuged for 30 minutes at 14,000 rpm. The mixture was passed through a SepPak (5g) cartridge (H_2O/methanol) and then purified on Biogel P4 (2x50cm) in 5% methanol to yield **89** (0.0285 g; 38 μmol; 88%): $[\alpha]_D$ –4 (c 0.73, H_2O); ^1H NMR (600 MHz, D_2O) δ 5.92 (ddd, 1H, H-4_{sph}, $J_{4,3}$ = 6.8 Hz, $J_{4,5a}$ = 17.2 Hz, $J_{4,5b}$ = 10.5 Hz), 5.39 (ddd, 1H, H-$5a_{sph}$, $J_{5a,5b} = J_{5a,3}$ = 1.3 Hz), 5.34 (4.53, d, 1H, H-$5b_{sph}$, $J_{5b,3}$ = 1.2 Hz), 4.53 (d, 1H, H-1', $J_{1',2'}$ = 7.9 Hz), 4.51 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 4.37-4.34 (m, 1H, H-3_{sph}), 4.11 (dd, 1H, H-3', $J_{3',2'}$ = 9.9 Hz, $J_{3',4'}$ = 3.2 Hz), 3.99 (dd, 1H, H-6a, $J_{6a,5}$ = 2.3 Hz, $J_{6a,6b}$ = 12.4 Hz), 3.96 (d, 1H, H-4'), 3.92-3.81 (m, 7H, H-$1a_{sph}$, H-2_{sph}, H-$1b_{sph}$, H-9"a, H-8", H-5", H-6b), 3.78-3.62 (m, 8H, H-6a', H-6b', H-5', H-4", H-4, H-3, H-9b", H-6"), 3.61-3.58 (m, 2H, H-7", H-5), 3.57 (dd, H, H-2'), 3.35 (dd, 1H, H-2, $J_{2,3}$ = 8.8 Hz), 2.76 (dd, 1H, H-3"eq, $J_{3e",4"}$ = 4.7 Hz, $J_{3e",3a"}$ = 12.4 Hz), 2.03 (*s*, 3H, C(O)CH_3), 1.80 (dd, 1H, H-3_{ax}", $J_{3a",4"}$ = 12.2 Hz); ^{13}C NMR (125 MHz, D_2O) δ 175.9, 174.7, 136.0, 119.6, 103.5, 102.9, 100.7, 79.1, 76.4, 76.1, 75.7, 75.2, 73.8, 73.6, 73.1, 72.7, 70.3, 69.5, 69.2, 69.0, 68.4, 65.8, 63.5, 61.9, 60.9, 52.6, 40.6, 22.9; ESI HRMS calc'd for $C_{28}H_{45}O_{20}N_4$: 757.2633. Found: 757.2626.

(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid-(2→3)-[2-acetamido-2-deoxy-*O*-(β-D-galactopyranosyl)-(1→4)]-*O*-(β-D-

galactopyranosyl)-(1→4)-*O*-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-azido-pent-4-ene-1,3-diol (90)

β-D-Galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*)-2-azido-pent-4-ene-1,3-diol[166] (0.050 g; 0.11 mmol) was combined with CMP-sialic acid (0.344 g; ~ 0.12 mmol; ~ 1.1 eq.). To this mixture was added successively α(2→3) sialyltransferase[217] in 50 mM HEPES (pH 7.5), containing 10% glycerol (9.52 mL; 0.14 U/mL), 0.5 M $MnCl_2$ (200 μL; 10 mM final conc.); 0.5 M $MgCl_2$ (200 μL; 10 mM final conc.); 0.4 M dithiothreitol (75 μL; 3 mM final conc.) and alkaline phosphatase (10 μL; 10U). The mixture was tumbled overnight and then centrifuged for 30 minutes at 14,000 rpm. The supernatant was loaded onto a SepPak cartridge (5 g) in water and eluted with a methanol-water gradient. The eluent was concentrated and combined with UDP-*N*-acetylglucosamine (0.134 g; 0.16 mmol; 1.5 eq.). To this mixture was added *N*-acetylgalactosaminyl transferase[213] (2.937 mL; 1.2U/mL) in 50 mM HEPES, pH 7.5 containing 10% glycerol (v/v) and UDP *N*-acetylglucosamine 4-epimerase[212] (2.937 mL; unknown activity) in 0.2 M NaCl, 1.0 mM EDTA, 20mM HEPES, 5 mM β-mercaptoethanol (pH 7.0) followed by 0.5M $MnCl_2$ (120 μL; 10 mM final conc.) and alkaline phosphatase (8 μL; 8 U). The mixture was tumbled gently overnight and then centrifuged for 20 min at 14,000 rpm. The supernatant was applied to a SepPak cartridge (5 g) and eluted with a gradient of methanol and water. The eluent was concentrated, redissolved in 5% methanol and purified on Biogel P4 (2x50cm) to yield **90** (0.0753 g; 0.08 mmol; 73%): $[\alpha]_D$ 10 (c 0.94, H_2O); ^1H NMR (600 MHz, D_2O) δ 5.93 (ddd, 1H, H-4_{sph}, $J_{4,3}$ = 6.9 Hz, $J_{4,5a}$ = 17.2 Hz, $J_{4,5b}$ = 10.6 Hz), 5.39 (d, 1H, H-$5a_{sph}$, $J_{5a,4}$ =17.2), 5.34 (4.53, d, 1H, H-$5b_{sph}$, $J_{5b,4}$ = 10.6 Hz), 4.75 (d, 1H, H-1‴, $J_{1''',2'''}$ = 8.6 Hz), 4.54 (d, 1H, H-1', $J_{1',2'}$ = 7.9 Hz), 4.52 (d, 1H, H-1, $J_{1,2}$ = 8.2 Hz), 4.36 (dd, 1H, H-

3_{sph}, $J_{3,4}$ = 6.5 Hz, $J_{3,2}$ = 4.8 Hz), 4.16 (dd, 1H, H-3', $J_{3',4'}$ = 3.0 Hz, $J_{3',2'}$ = 9.8 Hz), 4.12 (d, 1H, H-4'), 3.99 (dd, 1H, H-6a, $J_{6a,5}$ = 2.1 Hz, $J_{6a,6b}$ = 12.2 Hz), 3.95-3.85 (m, 6H, H-2''', H-1a_{sph}, H-9a'', H-2$_{sph}$, H-1b_{sph}, H-6a'''), 3.84-3.73 (m, 9H, H-5'', H-8'', H-9b'', H-6b, H-4'', H-6a', H-6b', H-5', H-4'''), 3.73-3.57 (m, 8H, H-3''', H6b''', H-5''', H-3, H-4, H-5'', H-7'', H-5), 3.49 (dd, 1H, H-6'', J = 1.9 Hz, J = 10.0 Hz), 3.37 (dd, 1H, H-2', $J_{2',3'}$ = 10.0 Hz), 3.35 (dd, 1H, H-2, $J_{2,3}$ = 9.5 Hz), 2.67 (dd, 1H, H-3''eq, $J_{3e'',4''}$ = 4.7 Hz, $J_{3e'',4''}$ = 12.7 Hz), 2.04 (s, 3H, C(O)CH_3), 2.02 (s, 3H, C(O)CH_3), 1.93 (dd, 1H, H-3''ax, $J_{3a'',4''}$ = 11.3 Hz); ^{13}C NMR (125 MHz, D_2O) δ 175.9, 175.9, 174.9, 136.0, 119.6, 103.6, 103.5, 102.9, 102.5, 79.4, 78.0, 75.7, 75.6, 75.2, 74.9, 73.9, 73.5, 73.14, 73.09 (2C), 72.2, 70.9, 69.6, 69.5, 68.9, 68.7, 65.8, 63.7, 62.0, 61.5, 60.9, 53.2, 52.5, 37.9, 23.5, 22.9; ESI HRMS calc'd for $C_{36}H_{58}N_5O_{25}$: 960.3426. Found: 960.3434.

6-Azidohexyl (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)-β-D-galactopyranoside (91)

6-Azidohexyl β-D-galactopyranoside[218] (0.017 g; 0.06 mmol) was combined with crude CMP-Neu5Ac (crude mass 180 mg; estimate 0.09 mmol; 1.5 eq.). α(2,3) Sialyl transferase (CST-04 in 50 mM HEPES, pH 7.5, 10% glycerol (v/v); 0.14U/mL; 4.75 mL), $MnCl_2$ and $MgCl_2$ (100 μL of a 0.5M solution; final conc. 10 mM), dithiothreitol (38 μL, 0.4M; final conc. 3mM) and alkaline phosphatase (12 μL) were added and the mixture was tumbled overnight. After centrifugation (30 min at 14,000 rpm) the supernatant was loaded onto a SepPak cartridge (5g) and eluted with a gradient of water and methanol to afford **91** (0.0311 g; 0.05 mmol; 94 %): $[\alpha]_D$ –3 (c 0.52, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 4.46 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 4.08 (dd, 1H, H-3, $J_{3,2}$ = 9.8 Hz, $J_{3,4}$ = 3.3 Hz), 3.95 (d, 1H, H-4, $J_{3,4}$ = 3.3 Hz), 3.94-3.85 (m, 3H, OCH_2, H-8', H-9a'), 3.84 (dd, 1H, H-5', $J_{5',6'}$ = $J_{5',6'}$ = 10.1 Hz), 3.77-3.72 (m, 2H, H-6a, H-6b), 3.72-3.61 (m, 5H,

H-4', H-5, OCH_2, H-6', H-9b'), 3.59 (dd, 1H, H-7', J = 1.8 Hz, J = 9.1 Hz), 3.53 (dd, 1H, H-2), 3.32 (dd, 2H, CH_2N$_3$), 2.76 (dd, 1H, H-3'$_{eq}$, $J_{3eq',4'}$ = 4.7 Hz, $J_{3eq',3ax'}$ = 12.4 Hz), 2.03 (s, 3H, C(O)CH_3), 1.80 (dd, 1H, H-3'$_{ax}$, $J_{3ax',4'}$ = 12.2 Hz), 1.67-1.58 (m, 4H, CH$_2$CH_2N$_3$, OCH$_2$CH_2), 1.43-1.37 (m, 4H, CH$_2$CH$_2$); ^{13}C NMR (125 MHz, D$_2$O) δ 175.9 (2C), 103.4, 100.7, 76.8, 75.7, 73.7, 72.6, 71.2, 70.0, 69.2, 69.0, 68.4, 63.5, 61.8, 52.6, 52.0, 40.6, 29.5, 28.8, 26.6, 25.5, 22.9; ESI HRMS calc'd for $C_{23}H_{39}N_4O_{14}$: 641.22527. Found: 641.22510.

6-Aminohexyl (5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(2→3)-β-D-galactopyranoside (92):

The azide **91** (0.024 g; 0.04 mmol) was combined with triphenylphosphine (0.053 g; 0.2 mmol; 5.0 eq.) and a mixture of pyridine and water (9:1, 5 mL) was added. The suspension was stirred at 65 °C for 36 hours, then diluted with water and filtered through a plug of sand and cotton. The filtrate was concentrated and then passed through a tC18 SepPak cartridge (0.4 g) preconditioned with ~1% NH_4OH before final purification by HPLC (Hamilton PRP-1 column, methanol-water gradient) to give **92** (0.0186 g; 0.03 mmol; 78 %): α_D –2.6 (c 0.40, H_2O). ^{1}H NMR (600 MHz, D$_2$O) δ 4.46 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 4.08 (dd, 1H, H-3, $J_{3,2}$ = 9.7 Hz, $J_{3,4}$ = 3.2 Hz), 3.95 (d, 1H, H-4, $J_{3,4}$ = 3.2 Hz), 3.93 (ddd, 1H, OCH_2, J = J = 3.1 Hz, J = 9.8 Hz), 3.90-3.86 (m, 2H, H-8', H-9a'), 3.84 (dd, 1H, H-5', $J_{5',6'}$ = $J_{5',6'}$ = 10.2 Hz), 3.77-3.62 (m, 7H, H-6a, H-6b, H-4', H-5, OCH_2, H-6', H-9b'), 3.60 (dd, 1H, H-7', J = 1.7 Hz, J = 9.1 Hz), 3.54 (dd, 1H, H-2), 3.00 (dd, 2H, CH_2NH$_2$), 2.76 (dd, 1H, H-3'$_{eq}$, $J_{3eq',4'}$ = 4.8 Hz, $J_{3eq',3ax'}$ = 12.5 Hz), 2.03 (s, 3H, C(O)CH_3), 1.80 (dd,1H, H-3'$_{ax}$, $J_{3ax,4'}$ = 12.0 Hz), 1.70-1.61 (m, 4H, CH$_2$CH_2NH$_2$, OCH$_2$CH_2), 1.45-1.39 (m, 4H, CH$_2$CH$_2$); ^{13}C NMR (125 MHz, D$_2$O) δ 175.9, 174.7,

103.4, 100.7, 76.8, 75.8, 73.8, 72.7, 71.1, 70.0, 69.2, 69.0, 68.4, 63.5, 61.9, 52.6, 52.0, 40.6, 40.3, 29.3, 27.6, 26.1, 25.4, 22.9; ESI HRMS calc'd for $C_{23}H_{42}N_2O_{14}$: 593.2528. Found: 593.2537.

Allyl *S*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonate)-(2→3)-2-*O*-benzoyl-3-thio-β-D-galactopyranoside (93)

Disaccharide **32** (0.285 g; 0.32 mmol) was dissolved in 80% acetic acid (10 mL) and the mixture was heated at 55 °C overnight with stirring. After evaporation of the volatiles, the residue was concentrated from toluene several times. Chromatography of the residue on silica gel using dichloromethane-methanol (96:4) as eluent yielded **93** (0.217 g; 0.27 mmol; 85%): $[\alpha]_D$ 22 (*c* 1.15, CHCl3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15-8.11 (m, 2H, Ar), 7.59-7.54 (m, 1H, Ar), 7.49-7.43 (m, 2H, Ar), 5.84-5.73 (m, 1H, H-b), 5.58 (ddd, 1H, H-8', J = 2.8 Hz, J = 5.7 Hz, $J_{8',7'}$ = 9.8 Hz), 5.24-5.16 (m, 3H, H-2, H-7', H-c), 5.10 (bd, 1H, N*H*), 5.05 (ddd, 1H, H-d, J = 1.4 Hz, J = 1.4 Hz, J = 1.4 Hz, J = 10.5 Hz), 4.97 (d, 1H, H-1, J = 7.7 Hz), 4.80 (ddd, 1H, H-4', $J_{4',3'eq}$ = 4.6 Hz, $J_{4',3'ax}$ = 11.7 Hz, $J_{4',5'}$ = 10.3 Hz), 4.37-4.29 (m, 2H, H-9'a, H-a), 4.15 (dddd, 1H, H-a', J = 1.4 Hz, J = 1.4 Hz, J = 5.9 Hz, J = 13.2 Hz), 4.06 (dd, 1H, H-9'b, $J_{9'b,8'}$ = 5.8 Hz, $J_{9'b,9'a}$ = 12.5 Hz), 3.95-3.83 (m, 4H, H-6a, H-6b, H-5, H-5'), 3.79-3.74 (m, 1H, H-6'), 3.77 (s, 3H, CO_2CH_3), 3.71 (dd, 1H, H-3, $J_{3,4}$ = 2.7 Hz, $J_{3,2}$ = 11.7 Hz), 3.62 (bd, 1H, H-4), 2.60 (dd, 1H, H-3'$_{eq}$, $J_{3eq,3ax}$ = 12.8 Hz), 2.4-2.2 (b, 2H, 2xOH), 2.19, 2.07, 1.98, (3s, 3 x 3H, 3 x C(O)CH_3), 1.94 (dd, 1H, H-3$_{ax}$), 1.83, 1.70 (2s, 2 x 3H, 2 x C(O)CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 170.8, 170.25, 170.20, 170.12, 169.4, 165.8, 133.9, 132.9, 130.3, 130.1, 128.3, 117.1, 101.4, 81.4, 75.2, 73.6, 70.3, 70.2, 69.3, 69.1, 67.4, 66.8, 63.2, 62.4, 53.2, 49.1, 48.2, 37.9, 23.1, 21.4, 20.8 (2C), 20.5; ESI HRMS calc'd for $C_{43}H_{51}NO_{18}SNa$: 836.2406.

Found: 836.2402. Anal. calc'd for $C_{36}H_{47}NO_{18}S$: C 53.13, H 5.82, N 1.72. Found: C 52.95, H 5.99, N 1.66.

A small amount of a side product, *S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonate)-(2→3)-2-*O*-benzoyl-3-thio-β-D-galactopyranosyl-(1→1)-2-propanone (**94**), was also isolated. (0.021 g; 0.03 mmol; 8%): $[\alpha]_D$ 31 (*c* 1.06, CHCl3); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.18-8.16 (m, 2H, Ar), 7.60-7.56 (m, 1H, Ar), 7.49-7.46 (m, 2H, Ar), 5.62 (ddd, 1H, H-8', $J_{8';7'}$ = 9.9 Hz, $J_{8';9a'}$ = 2.5 Hz, $J_{8';9b'}$ = 6.2 Hz), 5.28 (dd, 1H, H-2, $J_{2,1}$ = 7.6 Hz, $J_{2,3}$ = 11.8 Hz), 5.19 (dd, 1H, H-7', $J_{7';6'}$ = 2.3 Hz), 5.16 (d, 1H, N*H*Ac), 4.99 (d, 1H, H-1), 4.81 (ddd, 1H, H-4', $J_{4';3'ax}$ = 11.9 Hz, $J_{4';3'ax}$ = 4.6 Hz, $J_{4';5'}$ = 10.4 Hz), 4.36 (dd, 1H, H-9a', $J_{9a';9b'}$ = 12.4 Hz), 4.25 (d, 1H, $OCH_2C(O)CH_3$, *J* = 16.7 Hz), 4.16 (d, 1H, $OCH_2C(O)CH_3$, *J* = 16.7 Hz), 4.05 (dd, 1H, H-9b'), 3.94-3.84 (m, 4H, H-5, H-5', H-6a, H-6b), 3.78 (s, 3H, CO_2CH_3), 3.78-3.76 (m, 1H, H-6'), 3.74 (dd, 1H, H-3, $J_{3,4}$ = 2.7 Hz), 3.66-2.65 (m, 1H, H-4), 3.17 (d, 1H, O*H*), 2.60 (dd, 1H, H-3'$_{eq}$, $J_{3'eq,3'ax}$ = 12.8 Hz), 2.56-2.52 (m, 1H, O*H*), 2.19 (s, 3H, $NHC(O)CH_3$), 2.07 (s, 3H, $OCH_2C(O)CH_3$), 2.06 (s, 3H, $C(O)CH_3$), 1.98 (s, 3H, $C(O)CH_3$), 1.94 (dd, 1H, H-3'$_{ax}$), 1.84 (s, 3H, $C(O)CH_3$), 1.74 (s, 3H, $C(O)CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.4, 170.9, 170.8, 170.4, 170.22, 170.19, 169.4, 165.9, 133.1, 130.11, 130.07, 128.4, 102.0, 81.4, 75.3, 74.0, 73.6, 70.2, 69.3, 68.7, 67.4, 66.9, 62.8, 62.6, 53.2, 49.1, 48.0, 37.5, 26.8, 23.1, 21.4, 20.78, 20.77, 20.6; ESI HRMS calc'd for $C_{36}H_{47}NO_{19}SNa$: 852.2355. Found: 852.2355.

(2*S*,3*R*)-1-*S*-Acetyl-2-azido-1-thio-pent-4-ene-3-ol (95)

Diisopropyl azodicarboxylate (0.060 mL; 0.38 mmol; 1.1 eq.) was combined with triphenylphosphine (0.101 g; 0.39 mmol; 1.1 eq.) in tetrahydrofuran (2.0 mL) and the

mixture was stirred at 0 °C. Stirring was continued for 30 minutes before a solution of (2*S*,3*R*)-2-azido-1-pent-4-ene-1,3-diol[166]* (0.050 g; 0.35 mmol) and thiolacetic acid (0.026 mL; 0.37 mmol; 1.05 eq.) in tetrahydrofuran (1.5 mL) was added dropwise over one minute. After one hour, the solution was warmed to room temperature and stirring was continued for a further 60 hours. The mixture was concentrated and chromatographed on silica gel using hexanes/ethyl acetate (5:1) as eluent to afford **95** (0.054 g; 0.27 mmol; 77%): $[\alpha]_D$ –81 (c 0.84, CHCl3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.91 (ddd, 1H, H-4, J = 6.4 Hz, J = 10.5 Hz, J = 17.1 Hz), 5.39 (d, 1H, H-5a, $J_{5a,4}$ = 17.1 Hz), 5.32 (d, 1H, H-5b, $J_{5b,4}$ = 10.5 Hz), 4.20 (dd, 1H, H-3, $J_{3,2}$ = 5.8 Hz, $J_{3,4}$ = 5.8 Hz), 3.57 (ddd, 1H, H-2, $J_{2,1a}$ = 4.6 Hz, $J_{2,1b}$ = 8.0 Hz), 3.17 (dd, 1H, H-1a, $J_{1a,1b}$ = 14.2 Hz), 2.98 (dd, 1H, H-1b), 2.37 (s, 3H, SC(O)CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.7, 135.5, 118.7, 74.2, 65.7, 30.5, 30.0; ESI HRMS calc'd for $C_7H_{11}N_3O_2SNa$: 224.04642. Found: 224.04608. Anal. calc'd for $C_7H_{11}N_3O_2S$: C 41.78, H 5.51, N 20.88. Found: C 42.06, H 5.63, N 20.1; IR ($CHCl_3$ cast): ν_{OH} 3455 (m), ν_{N3} 2117 (s), ν_{CO} 1694 (s).

(2*S*,3*R*)-2-Azido-3-*O*-benzoyl-1-*O*-methanesulfonyl-pent-4-ene-diol (96)

(2*S*,3*R*)-2-Azido-3-*O*-benzoyl-pent-4-ene-diol[166] (0.198 g; 0.80 mmol) was combined with 4-(dimethylamino) pyridine and dissolved in dichloromethane (3.0 mL) and pyridine (0.130 mL; 1.60 mmol; 2.0 eq.) before cooling to 0 °C. Methanesulfonyl chloride (0.093 mL; 1.20 mmol; 1.5 eq.) was added, followed after 30 minutes by additional pyridine (0.300 mL) and methanesulfonyl chloride (0.200 mL; 2.5 mmol; 3.1 eq.). After stirring for a further one hour the mixture was concentrated and chromatographed on a column of silica gel using hexanes/ethyl acetate (3:1) as eluent to

* This material was prepared by Corwin Nycholat.

give **96** (0.251 g; 0.77 mmol; 96%): $[\alpha]_D$ –28 (c 1.02, CHCl3); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.03-8.01 (m, 2H, Ar), 7.60-7.56 (m, 1H, Ar), 7.47-7.44 (m, 2H, Ar), 5.95 (dddd, 1H, H-4, *J* = 0.9 Hz, *J* = 6.9 Hz, *J* = 10.5 Hz, *J* = 17.2 Hz), 5.65 (ddd, 1H, H-3, *J* = 1.1 Hz, *J* = 4.6 Hz, *J* = 6.9 Hz), 5.51 (dd, 1H, H-5a, *J* = 17.2 Hz, *J* = 1.0 Hz), 5.43 (dd, 1H, H-5b, *J* = 10.5 Hz, *J* = 1.0 Hz), 4.36 (ddd, 1H, H-1a, *J* = 1.1 Hz, *J* = 4.2 Hz, *J* = 10.9 Hz), 4.22 (ddd, 1H, H-1b, *J* = 1.1 Hz, *J* = 8.0 Hz, *J* = 10.9 Hz), 4.11-4.07 (m, 1H, H-2), 3.08 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.8, 133.5, 130.8, 129.8, 129.2, 128.5, 121.3, 73.9, 67.1, 62.7, 37.9; ESI HRMS calc'd for $C_{13}H_{15}N_3O_5SNa$: 348.0625. Found: 348.0629. Anal. calc'd for $C_{13}H_{15}N_3O_5S$: C 47.99, H 4.65, N 12.92. Found: C 48.02, H 4.48, N 12.82.

(2*S*,3*R*)-1-*S*-Acetyl-2-azido-3-*O*-benzoyl-1-thio-pent-4-ene-3-ol (97)

Mesylate **96** (0.055 g; 0.17 mmol) was combined with potassium thioacetate (0.096 g; 0.84 mmol; 5.0 eq.) in *N,N*-dimethylformamide. The mixture was immersed in an oil bath at 60 °C and stirred for 5 hours, then stirred overnight at room temperature. The mixture was concentrated, redissolved in dichloromethane (10 mL) and washed once with an equal volume of water. After drying over sodium sulfate and removal of the solvent, chromatography of the residue on a column of silica gel using hexanes/ethyl acetate (10:1) as eluent to give **97** (0.045 g; 0.15 mmol; 86%): $[\alpha]_D$ –81 (c 0.84, CHCl3); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.08-8.05 (m, 2H, Ar), 7.59-7.55 (m, 1H, Ar), 7.47-7.45 (m, 2H, Ar), 5.96 (ddd, 1H, H-4, $J_{4,3}$ = 6.9 Hz, $J_{4,5b}$ = 10.5 Hz, $J_{4,5a}$ = 17.2 Hz), 5.64 (dddd, 1H, H-3, $J_{3,5a}$ = 1.1 Hz, $J_{3,5b}$ = 1.1 Hz, $J_{3,2}$ = 4.1 Hz, $J_{3,2}$ = 6.9 Hz), 5.50 (ddd, 1H, H-5a, *J* = 1.1 Hz, *J* = 1.1 Hz, *J* = 17.2 Hz), 5.44 (ddd, 1H, H-5b, *J* = 1.1 Hz, *J* = 1.1 Hz, *J* = 10.5 Hz), 3.85 (ddd, 1H, H-2, $J_{2,3}$ = 4.2 Hz, $J_{2,1a}$ = 4.8 Hz, $J_{2,1b}$ = 8.9 Hz), 3.19 (dd, 1H,

H-1a, $J_{1a,1b}$ = 14.1 Hz), 2.90 (dd, 1H, H-1b), 2.37 (s, 3H, SC(O)CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.0, 165.0, 133.4, 131.0, 129.8, 129.5, 128.5, 121.2, 76.2, 64.3, 30.5, 30.0; ESI HRMS calc'd for $C_{14}H_{15}N_3O_3SNa$: 328.0726. Found: 328.0721. Anal. calc'd for $C_{13}H_{15}N_3O_3S$: C 55.07, H 4.95, N 13.76. Found: C 55.36, H 5.08, N 13.67.

(2*S*,3*R*)-2-Azido-3-thio-pent-4-ene-3-ol (98)

The benzoate **97** was dissolved in dry methanol and argon was bubbled through the solution for 30 minutes. A small piece of sodium metal was added and the reaction mixture was stirred overnight. After neutralization of the mixture with acidic ion exchange resin and removal by filtration, the solution was concentrated and the residue passed through a short plug of silica gel using hexanes/ethyl acetate (6:1) as eluent. Alternatively, treatment of **95** with hydrazine acetate (2 eq.) in degassed *N,N*-dimethylformamide, followed by concentration and chromatography afforded the same product. (Typical yields ~ 80%): $[\alpha]_D$ –58 (c 0.63, THF); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.89 (ddd, 1H, H-4, *J* = 6.6 Hz, *J* = 10.4 Hz, *J* = 17.1 Hz), 5.38 (d, 1H, H-5a, *J* = 17.1), 5.11 (d, 1H, H-5b, *J* = 10.4), 4.29 (bs, 1H, H-3), 3.54 (ddd, 1H, H-2, *J* = 5.1 Hz, *J* = 5.1 Hz, *J* = 7.6 Hz), 2.72-2.64 (m, 2H, H-1a, H-1b), 1.93 (bs, 1H, OH), 1.61 (dd, 1H, SH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 135.8, 118.8, 74.2 68.6, 25.4; IR ($CHCl_3$ cast): ν_{OH} 3372 (m), ν_{N3} 2116 (s). After standing for several weeks at 4 °C, the solid material was observed to darken in colour. Elemental analysis did not correspond to the title compound. Analysis of the substance by ^{1}H NMR revealed a mixture of compounds, including what appeared to be some of the target structure obtained originally. ESI HRMS calc'd for $C_5H_{12}NOSNa$: 134.0634. Found: 134.0636, and $C_{10}H_{19}N_4O_2S_2$: 291.0944. Found 291.0947.

Octyl (2,4,6-tri-*O*-acetyl-3-*S*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (99)

Octaacetate **37** (0.272 g; 0.39 mmol) was dissolved in dry dichloromethane (10.0 mL) and 1-octanol (0.066 mL; 0.44 mmol; 1.1 eq.) containing activated 3Å molecular sieves (0.20 g) and the mixture was stirred under an argon atmosphere for one hour. The reaction mixture was cooled to 0 °C and a solution of $SnCl_4$ (0.057 mL; 0.49 mmol) in dichloromethane (2.0 mL) was added dropwise. After 3 hours, the reaction mixture was neutralized with pyridine, diluted with dichloromethane (20 mL), and washed successively with 1M HCl, saturated $NaHCO_3$ solution, and water. The organic layers were dried over Na_2SO_4, filtered, and concentrated. Column chromatography of the residue on silica gel, using hexanes-ethyl acetate (5:3) as eluent afforded compound **99** (0.195 g; 0.25 mmol; 65%). $[\alpha]_D = 6$ (*c* 0.41, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.23 (d, 1H, H-4', $J_{4',3'} = 3.1$ Hz), 5.15 (dd, 1H, H-3, $J_{3,2} = J_{3,4} = 9.3$ Hz), 4.92 (dd, 1H, H-2', $J_{2',1'} = 7.8$ Hz, $J_{2',3'} = 11.8$ Hz), 4.86 (dd, 1H, H-2, $J_{2,1} = 8.0$ Hz), 4.51 (d, 1H, H-1'), 4.46 (dd, 1H, H-6a, $J_{6a,6b} = 11.9$ Hz, $J_{6a,5} = 2.0$ Hz), 4.42 (d, 1H, H-1), 4.07 (dd, 1H, H-6b), 4.00 (m, 2H, H-6a', H-6b'), 3.95 (m, 1H, H-5'), 3.84 (dd, 1H, H-3'), 3.78 (m, 2H, H-4, $OCH_2C_7H_{15}$), 3.57 (m, 1H, H5), 3.42 (m, 1H, $OCH_2C_7H_{15}$), 2.28, 2.11, 2.10, 2.04, 2.01, (5s, 15H, 5x$C(O)CH_3$), 2.00 (s, 6H, 2x $C(O)CH_3$), 1.57-1.47 (m, 2H, $OCH_2CH_2C_6H_{13}$), 1.30-1.20 (m, 10H, $OCH_2CH_2C_5H_{10}CH_3$), 0.85 (t, 3H, CH_3); ^{13}C NMR (125MHz $CDCl_3$) δ 193.7, 170.3 (2C), 169.8, 169.7, 169.5, 169.2, 102.1, 100.6, 76.1, 73.2, 72.8, 72.6, 71.7, 70.2, 68.2, 67.9, 62.1, 61.4, 47.2, 31.8, 30.5, 29.4, 29.23, 29.21, 25.8, 22.6, 20.83, 20.77, 20.64, 20.62, 20.56, 20.53, 14.0; ESI HRMS calc'd for $C_{34}H_{52}O_{17}SNa$: 787.282292. Found: 787.282150. Anal. calc'd for $C_{34}H_{52}O_{17}S$: C 53.39, H 6.85. Found: C 53.74, H 7.05.

Octyl (3-thio-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (100)

Glycoside **99** (0.060 g; 0.08 mmol) was dissolved in anhydrous methanol (6.0 mL) with stirring. After bubbling argon through the solution for 30 minutes, several drops of 0.5 M sodium methoxide solution were added. Stirring was continued under an argon atmosphere overnight, then the solution was neutralized with acidic ion exchange resin. Following filtration, the solution was concentrated, and the residue suspended in deoxygenated water (~ 5 mL). The suspension was passed through a Waters Sep-Pak C18plus cartridge, and the cartridge was rinsed with 15 mL deoxygenated water, then eluted with HPLC grade methanol (10.0 mL). The methanol-containing eluate was concentrated, and the solid obtained was lyophilized from water to afford **100** (34 mg; 93%). ^{1}H NMR (500 MHz, D_2O) δ 4.51-4.46 (m, 2H, H-1', H-1), 3.99 (dd, 1H, H-6a, $J_{6a,6b}$ = 12.2 Hz, $J_{6a,5}$ = 2.1 Hz), 3.94-3.87 (m, 2H, OCH_2C$_7$H$_{15}$, H-4'), 3.83-3.74 (m, 4H, H-6b, H-5', H-6a', H-6b'), 3.70-3.63 (m, 4H, H-3, H-4, OCH_2C$_7$H$_{15}$), 3.61-3.57 (m, 1H, H-5), 1.52 (m, 2H, OCH$_2$CH_2C$_6$H$_{13}$), 1.40-1.20 (m, 10H, OCH$_2$CH$_2$C$_5H_{10}$CH$_3$), 0.94 (t, 3H, CH_3); ESI HRMS calc'd for $C_{20}H_{38}O_9SNa$: 493.2083. Found: 493.2084.

Octyl (3-thio-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside disulfide (101)

Solutions of **100** proved sensitive to disulfide formation despite efforts to exclude oxygen. To facilitate characterization, a stirring solution of **100** in methanol was exposed to air overnight, resulting in quantitative conversion to the disulfide **101**. $[\alpha]_D$ 97 (*c* 0.28, MeOH). ^{1}H NMR (500 MHz, D_2O) δ 4.40 (d, 1H, H-1', $J_{1',2'}$ = 7.6 Hz), 4.27 (d, 1H, H-1, $J_{1,2}$ = 7.9 Hz), 4.12 (d, 1H, H-4', $J_{4',3'}$ = 2.8 Hz), 3.86 (m, 3H, H-6a, H-6b, OCH_2C$_7$H$_{15}$), 3.75 (dd, 1H, H-6a', $J_{6a',6b'}$ = 11.4 Hz, $J_{6a',5'}$ = 7.4 Hz), 3.69 (dd, 1H, H-6b', $J_{6b',5'}$ = 4.6 Hz), 3.63 (m, 1H, H-5'), 3.58 (dd, 1H, H-4, $J_{4,3} \approx J_{4,5} \approx$ 9.2 Hz), 3.56-

3.48 (m, 3H, H-2', H-3, OC*H*$_2$C$_7$H$_{15}$), 3.38 (ddd, 1H, H-5), 3.23 (dd, 1H, H-2, $J_{2,3}$ = 9.2 Hz), 3.09 (dd, 1H, H-3', $J_{2,3}$ = 11.5 Hz), 1.61 (m, 2H, OCH$_2$C*H*$_2$C$_6$H$_{13}$), 1.41-1.22 (m, 10H, OCH$_2$CH$_2$C$_5$*H*$_{10}$CH$_3$), 0.95 (t, 3H, C*H*$_3$); ^{13}C NMR (125MHz, D$_2$O) δ 106.3, 104.3, 80.5, 79.4, 76.51, 76.49, 74.8, 71.0, 70.4, 68.8, 62.7, 62.2, 62.0, 33.0, 30.8, 30.6, 30.4, 27.1, 23.7, 14.4; ESI HRMS calc'd for $C_{40}H_{74}O_{20}S_2Na$: 961.4113. Found: 961.4111. Anal. calc'd for $C_{40}H_{74}O_{20}S_2 \cdot 2H_2O$: C 49.27, H 8.06. Found: C 49.22, H 8.07.

(2,4,6-Tri-*O*-acetyl-3-*S*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-D-glucopyranoside (102)

The disaccharide **37** (0.200 g; 0.29 mmol) was concentrated from toluene (2x5 mL) and dissolved in dichloromethane (10.0 mL). The solution was cooled to 0 °C before adding hydrogen bromide in acetic acid (2.5 mL; 45% w/w). After stirring for approximately 4 hours, the solution was diluted with toluene (25 mL) and concentrated. The residue was taken up in a mixture of acetone (5.0 mL) and water (1.0 mL) and then silver carbonate (0.58 mmol; 2.0 eq.) was added. The reaction mixture was protected from the light and stirred for 7 hours, then filtered through celite, diluted with dichloromethane (30 mL), washed with water, dried over sodium sulfate, filtered, and concentrated. Chromatography of the residue on silica gel using hexanes-ethyl acetate (1:1→2:3) afforded **102** as a mixture of anomers (0.159 g; 0.24 mmol; 85%). Data for the α anomer: ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.51 (dd, 1H, H-3, $J_{3,2} \sim J_{3,4} \sim 9.9$ Hz), 5.38 (dd, 1H, H-1, $J_{1,2} \sim J_{1,OH\text{-}1} = 3.7$ Hz), 5.26 (bd, 1H, H-4', $J_{4',3'} = 3.1$ Hz), 4.97 (dd, 1H, H-2', $J_{2',1'} = 7.7$ Hz, $J_{2',3'} = 11.8$ Hz), 4.84 (dd, 1H, H-2), 4.56 (d, 1H, H-1'), 4.54-4.49 (m, 1H, H-6a), 4.19-3.96 (m, 5H, H-5, H-6b, H-6a', H-6b', H-5'), 3.87 (dd, 1H, H-3'), 3.77 (dd, 1H, H-4, $J_{4,5} = 9.8$ Hz), 2.97 (d, 1H, OH-1), 2.30, 2.142, 2.137, 2.08, 2.07 (5s, 15H,

5xC(O)CH_3), 2.04 (s, 6H, 2xC(O)CH_3); ^{13}C NMR (125MHz, $CDCl_3$) δ 193.9, 170.45, 170.39, 170.2, 169.9, 169.6, 169.2, 102.0, 90.2, 76.0, 73.2, 71.3, 69.5, 68.4, 68.2, 68.0, 61.9, 61.5, 47.3, 30.5, 20.9, 20.8, 20.73, 20.66, 20.59, 20.56; ESI HRMS calc'd for $C_{26}H_{36}O_{17}SNa$: 675.1565. Found: 675.1567; Anal. calc'd for $C_{26}H_{36}O_{17}S$: C 47.85, H 5.56. Found: C 47.69, H 5.83.

Octyl (α-D-galactopyranosyl)-(1→3)-(α-D-galactopyranosyl)-(1→3)-*S*-(3-thio-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (103)

The components of a 2.0 mL mixture of acceptor **100** (3.2 mg, 6.9 umol, 3.4 mM in reaction), UDP-Gal (dipotassium salt, 13.0 mg, 20.2 umol, 10 mM in reaction), $MnCl_2$ (2 mM), α1,3-GalT (1.0 mL, 12 U/mL), dithiothreitol (10 mM in reaction), BSA (0.2 mg), alkaline phosphatase (6 ul, 1 U/ul) in MOPS buffer (62 mM, pH 7.5) were combined in a 15 mL Falcon tube. The mixture was tumbled gently at room temperature for 2 days, after which time TLC ($CHCl_3$/MeOH/H_2O/AcOH, 6:3:1:1) indicated complete disappearance of thiol **100** and disulfide **101**. The reaction mixture was diluted with H_2O (3.0 mL), and purified by solid phase extraction on a SepPak (0.4 g) cartridge to yield **103** (5.1 mg; 6.4 umol; 93%) after lyophilization. $[\alpha]_D$ = 3 (*c* 0.055, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 5.61 (d, 1H, H-1", $J_{1",2"}$ = 5.6 Hz), 5.17 (d, 1H, H-1"', $J_{1"',2"'}$ = 4.0 Hz), 4.53 (d, 1H, H-1', $J_{1',2'}$ = 7.5 Hz), 4.48 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 4.36 (m, 1H, H-5"), 4.31 (dd, 1H, H-2", $J_{2",3"}$ = 10.4 Hz), 4.27 (m, 1H, H-4"), 4.17 (m, 1H, H-5"'), 4.04 (d, 1H, H-4', $J_{4',3'}$ = 2.6 Hz)), 4.01 (d, 1H, H-4"', $J_{4"',3"'}$ = 3.1 Hz), 3.98 (dd, 1H, H-6a, $J_{6a,6b}$ = 12.3 Hz, $J_{6a,5}$ = 1.9 Hz), 3.93 (m, 3H, H-3', H-3", OCH_2CH_2Si(CH3)3), 3.87 (dd, 1H, H-2"', $J_{2"',3"'}$ = 10.3 Hz), 3.76 (m, 8H, H-5', H-6a', H-6b', H-6"a, H-6"b, H-6b, H-6"'a, H-6"'b), 3.65 (m, 3H, H-3, H-4, OCH_2CH_2Si(CH3)3), 3.59 (m, 1H, H-5), 3.52 (1H,

dd, H-2', $J_{2',3'}$ = 11.4 Hz), 3.30 (dd, 1H, H-2, $J_{2,3}$ = 8.5 Hz), 3.15 (dd, 1H, H-3', $J_{3',4'}$ = 2.7 Hz), 1.63 (m, 2H, $OCH_2CH_2Si(CH3)3$), 1.32 (m, 10H, $OCH_2CH_2C_5H_{10}CH_3$), 0.86 (t, 3H, CH_3); ^{13}C NMR (125MHz, CD_3OD) δ 106.3, 104.3, 97.6, 85.4, 80.9, 79.2, 77.9, 76.53, 76.46, 74.8, 73.0, 72.4, 71.4, 71.2, 71.0, 70.6, 70.2, 68.4, 68.3, 67.5, 62.86, 62.85, 62.82, 62.1, 53.2, 33.0, 30.8, 30.6, 30.4, 27.1, 23.7, 14.4; $^1J_{C1,H1}$ (D_2O): ($^1J_{C1',H1'}$ = 162.4 Hz), ($^1J_{C1,H1}$ = 160.5 Hz), ($^1J_{C1''',H1'''}$ = 170.0 Hz) ($^1J_{C1'',H1''}$ = 166.9 Hz); ESI HRMS calc'd for $C_{32}H_{58}O_{20}SNa$: 817.3134. Found: 817.3131.

Octyl (α-D-galactopyranosyl)-(1→3)-*S*-(3-thio-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (104)

Tetrasaccharide **103** (2.70 mg; 3.4 umol) was dissolved in citrate buffer (1.5 mL; pH 6.0), and green coffee bean α-galactosidase [E.C. 3.2.1.22] (10 ul; 0.5 U) was added. The mixture was shaken gently at 37 °C for 2 hours, then diluted with 3.0 mL of water and and purified by solid phase extraction on a SepPak C_{18} cartridge (0.4 g) to yield **4.6** (2.06 mg; 3.3 umol; 96%). $[\alpha]_D$ 1 (*c* 0.05, H_2O). ^1H NMR (600 MHz, D_2O) δ 5.50 (d, 1H, H1", $J_{1'',2''}$ = 5.76 Hz), 4.45 (d, 1H, H1', $J_{1',2'}$ = 7.6 Hz), 4.40 (d, 1H, H1, $J_{1,2}$ = 8.1 Hz), 4.28 (m, 1H, H5"), 4.07 (dd, 1H, H2", $J_{2'',3''}$ = 10.3 Hz), 3.96 (d, 1H, H4', $J_{4',3'}$ = 2.8 Hz), 3.93 (d, 1H, H4", $J_{4'',3''}$ = 3.3 Hz, $J_{4'',5''}$ = 0.6 Hz), 3.90 (dd, 1H, H6a, $J_{6a,6b}$ = 12.3 Hz, $J_{6a,5}$ = 2.2 Hz), 3.84 (m, 1H, $OCH_2C_7H_{15}$), 3.74-3.67 (m, 7H, H6b, H5', H6a', H6b', H3", H6"a, H6"b), 3.70-3.62 (m, 3H, H3, H4, $OCH_2C_7H_{15}$), 3.51 (m, 1H, H5), 3.44 (dd, 1H, H2', $J_{2',3'}$ = 11.4 Hz), 3.21 (dd, 1H, H2, $J_{2,3} \approx 9$ Hz), 3.06 (dd, 1H, H3'), 1.54 (m, 2H, $OCH_2CH_2C_6H_{13}$), 1.32-1.15 (m, 10H, $OCH_2CH_2C_5H_{10}CH_3$), 0.78 (t, 3H, CH_3); ^{13}C NMR (125MHz, CD_3OD) δ 106.3, 104.3, 85.2, 80.9, 79.2, 76.52, 76.46, 74.8, 73.3, 72.3, 70.99, 70.97, 70.6, 69.7, 68.4, 62.9, 62.8, 62.1, 53.2, 33.0, 30.8, 30.6, 30.4, 27.1, 23.7, 14.4;

$^1J_{C1,H1}$ (D_2O): ($^1J_{C1',H1'}$ = 162.5 Hz), ($^1J_{C1,H1}$ = 161.8 Hz), ($^1J_{C1'',H1''}$ = 167.1 Hz). ESI HRMS calc'd for $C_{26}H_{48}O_{15}SNa$: 655.2612. Found: 655.2611.

Octyl (2-acetamido-2-deoxy-β-D glucopyranosyl)-(1→3)-*S*-3-thio-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (105)

To a solution of β1,3-GlcNAcT[249] (1.1 mL, 0.12 U/mL) in sodium cacodylate (25 mM; pH 7.5) containing $MnCl_2$ (15 mM), Triton X-100 (0.1%), and glycerol (2% v/v) were added acceptor **100** (1.0 mg, 2.1 umol, 1.9 mM in reaction), UDP-GlcNAc (disodium salt, 2.8 mg, 4.1 umol, 3.8 mM in reaction), dithiothreitol (24.2 ul of a 0.1M solution in MOPS buffer, pH 7.5; 2.2 mM in reaction), BSA (2.0 mg), and alkaline phosphatase (2 ul, 1 U/ul). The mixture was tumbled gently in a 1.5 mL Eppendorph tube at room temperature for 36 hours, after which time a radiochemical assay indicated negligible enzyme activity. The reaction mixture was diluted with H_2O (3.0 mL), and loaded onto a SepPak cartridge (0.4 g). The compounds that were retained were eluted with a gradient of aqueous methanol (10 – 100% MeOH) to avoid contamination by Triton X-100. ESI HRMS calc'd for $C_{28}H_{51}O_{15}SNa$ 696.2872 Found: 696.2870.

8-Methoxycarbonyloctyl (2,3-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (106)

8-Methoxycarbonyloctyl (2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside[225] (2.144 g; 1.91 mmol) was dissolved in dichloromethane (50 mL), the solution was cooled to 0 °C, and aqueous (90%) trifluoroacetic acid (6 mL) was added. After thirty minutes the mixture was diluted with dichloromethane (50 mL) and washed with water (50 mL), saturated sodium bicarbonate solution (50 mL), water (50 mL) and then dried over sodium sulfate, filtered and

concentrated. The residue was recrystallized from hexanes-ethyl acetate to afford **106** (1.620 g; 1.74 mmol; 91%): $[\alpha]_D$ 72 (c 0.96, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 8.05-8.02 (m, 2H, Ar), 7.98-7.89 (m, 8H, Ar), 7.63-7.49 (m, 3H, Ar), 7.48-7.32 (m, 10H, Ar), 7.25-7.21 (m, 2H, Ar), 5.74 (dd, 1H, H-3, $J_{3,2} = J_{3,4} = 9.2$ Hz), 5.73 (dd, 1H, H-2', $J_{2',1'} = 7.8$ Hz, $J_{2',3'} = 10.3$ Hz), 5.40 (dd, 1H, H-2, $J_{2,1} = 7.8$ Hz, $J_{2,3} = 9.5$ Hz), 5.07 (dd, 1H, H-3', $J_{3',4'} = 3.1$ Hz), 4.79 (d, 1H, H-1'), 4.67 (d, 1H, H-1), 4.61 (dd, 1H, H-6a, $J_{6a,5} = 2.1$ Hz, $J_{6a,6b} = 11.9$ Hz), 4.42 (dd, 1H, H-6b, $J_{6b,5} = 5.0$ Hz), 4.22-4.16 (m, 2H, H-4, H-4'), 3.85 (ddd, 1H, H-5, $J_{5,4} = 9.7$ Hz), 3.78 (ddd, 1H, OCH_2, $J = 6.3$ Hz, $J = 6.3$ Hz, $J = 9.8$ Hz), 3.65 (s, 3H, CO_2CH_3), 3.44-3.34 (m, 3H, OCH_2, H-5', H-6a'), 3.29 (ddd, 1H, H-6b', $J = 5.1$ Hz, $J = 6.9$ Hz, $J = 11.5$ Hz), 2.75 (d, 1H, OH-4', $J = 4.4$ Hz), 2.21 (t, 2H, $CH_2CO_2CH_3$, $J = 7.5$ Hz), 1.56 (d, 1H, OH-6', $J = 6.9$ Hz), 1.53-1.37 (m, 4H, aglycon), 1.16-0.98 (m, 8H, aglycon); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.2, 165.9, 165.8, 165.4, 165.2, 165.1, 133.4, 133.3, 133.19, 133.16, 133.15, 129.83, 129.81, 129.74, 129.67, 129.65, 129.57, 129.4, 129.0, 128.86, 128.85, 128.6, 129.43, 128.38, 128.37, 128.32, 101.3, 100.9, 76.5, 74.3, 74.2, 73.6, 72.9, 71.9, 70.1, 69.7, 68.2, 62.6, 62.4, 51.4, 34.0, 29.3, 28.98, 28.96, 28.94, 25.7, 24.8; ESI HRMS calc`d for $C_{57}H_{60}O_{18}Na$: 1055.3680. Found: 1055.3677. Anal. calc`d for $C_{57}H_{60}O_{18}$: C 66.27, H 5.85. Found: C 66.18, H 5.61.

8-Methoxycarbonyloctyl (2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (107)

The diol **106** (0.950 g; 0.92 mmol) and 4-(dimethylamino)pyridine (10 mg) were combined in pyridine (2.0 mL) and dry dichloromethane (10.0 mL) under an argon atmosphere, and the solution was cooled to –78 °C. Benzoyl chloride (0.130 mL; 1.12

mmol; 1.2 eq.) was added dropwise over approximately one minute, and the mixture was allowed to warm to room temperature over two hours before methanol (2 mL) was added. After stirring for a further thirty minutes, the reaction mixture was concentrated and chromatographed using hexanes-ethyl acetate (2:1) as eluent to afford **107** (0.944 g; 0.83 mmol; 90%): $[\alpha]_D$ 45 (c 0.60, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.05-8.00 (m, 4H, Ar), 7.98-7.93 (m, 6H, Ar), 7.93-7.90 (m, 2H, Ar), 7.63-7.59 (m, 1H, Ar), 7.57-7.53 (m, 1H, Ar), 7.52-7.47 (m, 5H, Ar), 7.42-7.33 (m, 10H, Ar), 7.29-7.26 (m, 1H, Ar), 5.76 (dd, 1H, H-3, $J_{3,2} = J_{3,4} = 9.6$ Hz), 5.69 (dd, 1H, H-2', $J_{2',1'} = 7.8$ Hz, $J_{2',3'} = 10.3$ Hz), 5.41 (dd, 1H, H-2, $J_{2,1} = 7.9$ Hz), 5.12 (dd, 1H, H-3', $J_{3',4'} = 3.3$ Hz), 4.78 (d, 1H, H-1'), 4.65 (d, 1H, H-1), 4.59 (dd, 1H, H-6a, $J_{6a,5} = 1.9$ Hz, $J_{6a,6b} = 12.0$ Hz), 4.45 (dd, 1H, H-6b, $J_{6b,5} = 4.5$ Hz), 4.21 (dd, 1H, H-4, $J_{4,5} = 9.5$ Hz), 4.10-4.05 (m, 2H, H-4', H-6a'), 3.84-3.76 (m, 2H, H-5, OCH_2), 3.68-3.60 (m, 2H, H-5', H-6b'), 3.66 (s, 3H, CO_2CH_3), 3.41 (ddd, 1H, OCH_2, $J = 6.5$ Hz, $J = 6.5$ Hz, $J = 9.7$ Hz), 2.22(t, 2H, $CH_2CO_2CH_3$, $J = 7.5$ Hz), 2.15 (bs, 1H, OH-4'), 1.52-1.36 (m, 4H, aglycon), 1.18-0.97 (m, 8H, 4 x CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.2, 166.0, 165.8, 165.7, 165.5, 165.2, 164.9, 133.6, 133.43, 133.23, 133.21, 133.19, 133.09, 130.2, 129.85, 129.83, 129.75, 129.72, 129.64, 129.62, 129.58, 129.50, 129.47, 128.9, 128.8, 128.6, 128.47, 128.44, 128.42, 128.37, 128.31, 101.1, 101.0, 76.2, 74.0, 73.1, 73.0, 72.5, 71.9, 70.2, 69.6, 66.8, 62.5, 61.6, 51.4, 36.9, 29.3, 28.98, 28.97, 28.94, 25.7, 24.8; ESI HRMS calc`d for $C_{64}H_{64}O_{19}Na$: 1159.3940 Found: 1159.3937. Anal. calc`d for $C_{64}H_{64}O_{19}$: C 67.60, H 5.67. Found: C 67.69, H 5.54.

8-Methoxycarbonyloctyl (2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (108)

The lactoside **107** (0.831 g; 0.73 mmol) was dissolved in dichloromethane (10.0 mL) and pyridine (0.6 mL). After cooling the solution to –70°C, trifluoromethanesulfonic anhydride (0.370 mL; 2.20 mmol; 3 eq.) was added and the reaction mixture was allowed to warm to 10°C over a period of 1.5 hours. After dilution with dichloromethane (20 mL) the solution was washed with water (30 mL), dried over sodium sulfate, filtered and concentrated. The residue was dissolved in *N,N*-dimethylformamide (6 mL) and tetrabutylammonium nitrite (0.632 g; mmol; 3.0 eq.) was added. The solution was stirred overnight at 40 °C and then concentrated. Chromatography of the residue on silica gel using hexanes-ethyl acetate (2:1) as eluent afforded **108** (0.589 g; 0.52 mmol; 71%): $[\alpha]_D$ 35 (c 0.98, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.00-7.87 (m, 12H, Ar), 7.63-7.55 (m, 2H, Ar), 7.53-7.45 (m, 4H, Ar), 7.43-7.30 (m, 8H, Ar), 7.29-7.18 (m, 4H, Ar), 5.78 (dd, 1H, H-3, $J_{3,2} = J_{3,4} = 9.2$ Hz), 5.42 (dd, 1H, H-2', $J_{2',1'} = 7.9$ Hz, $J_{2',3'} = 9.5$ Hz), 5.37 (dd, 1H, H-2, $J_{2,1} = 7.9$ Hz), 5.28 (dd, 1H, H-3', $J_{3',4'} = 9.5$ Hz), 4.83 (d, 1H, H-1'), 4.65 (d, 1H, H-1), 4.59 (dd, 1H, H-6a, $J_{6a,5} = 1.9$ Hz, $J_{6a,6b} = 12.1$ Hz), 4.46 (dd, 1H, H-6b, $J_{6b,5} = 4.5$ Hz), 4.21 (dd, 1H, H-4, $J_{4,5} = 9.5$ Hz), 4.14 (dd, 1H, H-6a', $J_{6a',5'} = 2.1$ Hz, $J_{6a',6b'} = 12.1$ Hz), 4.07 (dd, 1H, H-6b', $J_{6b',5'} = 4.9$ Hz), 3.84-3.74 (m, 2H, H-5, OCH_2), 3.66 (s, 3H, CO_2CH_3), 3.68-3.60 (m, 1H, H-4'), 3.44 (ddd, 1H, H-5', $J_{5',4'} = 9.8$ Hz), 3.39 (ddd, 1H, OCH_2, $J = 6.6$ Hz, $J = 6.6$ Hz, $J = 9.8$ Hz), 3.20 (bs, 1H, OH-4'), 2.21 (t, 2H, CH_2CO_2Me, $J = J = 7.6$ Hz), 1.54-1.38 (m, 4H, 2 x CH_2), 1.16-0.98 (m, 8H, 4 x CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.4, 167.3, 166.5, 165.8, 165.3, 165.1, 164.9, 133.5, 133.4, 133.3, 133.21, 133.16, 133.05, 129.94, 129.82, 129.74, 129.71, 129.64, 129.61, 129.60, 129.59, 129.48, 129.44, 128.7, 128.6, 128.5, 128.44, 128.42, 128.37, 128.27, 128.25, 100.94, 100.89, 76.59, 76.56, 74.66, 73.1, 73.0, 72.1, 71.4, 70.1, 69.3, 62.8, 62.3,

51.3, 34.0, 29.3, 28.97, 28.96, 28.94, 25.6, 24.9 ; ESI HRMS calc'd for $C_{64}H_{64}O_{19}Na$: 1159.3940 Found: 1159.3939. Anal. calc'd for $C_{64}H_{64}O_{19}$: C 67.60, H 5.67. Found: C 67.79, H 5.64.

8-Methoxycarbonyloctyl (4-*S*-acetyl-2,3,6-tri-*O*-benzoyl-4-thio-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (109)

The cellobioside **108** (0.075 g; 0.07 mmol) was dissolved in freshly distilled dichloromethane (4.0 mL) and pyridine (0.066 mL), and was then cooled to –78 °C under an argon atmosphere. Trifluoromethanesulfonic anhydride (0.056 mL; 0.26 mmol; 4 eq.) was added, and the reaction mixture was allowed to warm to room temperature over a period of one hour. Stirring was continued for a further one hour, after which time the mixture was diluted with dichloromethane (20 mL), washed with water (2 x 10 mL), dried over sodium sulfate, filtered, and concentrated. The residue was combined with potassium thioacetate (0.020 g; 1.8 mmol; 2.6 eq.) in *N,N*-dimethylformamide (3.0 mL) and heated at 40 °C for two hours, then concentrated. The residue was dissolved in dichloromethane (25 mL), washed with water (2 x 15 mL), dried over sodium sulfate, filtered, and concentrated. Chromatography on a column of silica gel topped with a 2cm plug of activated carbon using a gradient of hexanes-ethyl acetate (10:5→8:5) as eluent afforded **109** (0.070 g; 0.06 mmol; 89%): $[\alpha]_D$ 4 (c 0.79, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 8.08-8.03 (m, 4H, Ar), 7.99-7.93 (m, 6H, Ar), 7.86-7.83 (m, 2H, Ar), 7.63-7.59 (m, 1H, Ar), 7.56-7.44 (m, 6H, Ar), 7.42-7.29 (m, 11H, Ar), 5.73 (dd, 1H, H-3, $J_{3,2} = J_{3,4}$ = 9.5 Hz), 5.43 (dd, 1H, H-2, $J_{2,1}$ = 7.9 Hz), 5.40-5.36 (m, 2H, H-2′, H-3′), 4.75 (m, 1H, H-1′),), 4.64 (d, 1H, H-1), 4.56 (dd, 1H, H-6a, $J_{6a,5}$ = 1.9 Hz, $J_{6a,6b}$ = 12.1 Hz), 4.44 (dd, 1H, H-6b, $J_{6b,5}$ = 4.4 Hz), 4.40 (m, 1H, H-4'), 4.19 (dd, 1H, H-4, $J_{4,5}$ = 9.5 Hz), 3.96-3.92

(m, 1H, H-5'), 3.86 (dd, 1H, H-6a', $J_{6a',5'}$ = 5.9 Hz, $J_{6a',6b'}$= 11.5 Hz), 3.83-3.77 (m, 2H, H-5, OCH_2), 3.65 (s, 3H, CO_2CH_3), 3.62 (dd, 1H, H-6b', $J_{6b',5'}$ = 7.0 Hz), 3.40 (ddd, 1H, OCH_2, J = 6.7 Hz, J = 6.7 Hz, J = 9.8 Hz), 2.22 (t, 2H, CH_2CO_2Me, J = 7.5 Hz), 2.14 (s, 3H, SC(O)CH_3), 1.52-1.37 (m, 4H, 2 x CH_2), 1.17-0.95 (m, 8H, 4 x CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.7, 174.2, 165.8, 165.6, 165.4, 165.2, 165.1, 164.7, 133.4, 133.34, 133.31, 133.2, 133.1 (2C), 130.0, 129.9, 129.8, 129.7, 129.62, 129.61 (2C), 129.56 (2C), 129.54, 129.46, 129.0, 128.71, 128.67, 128.51, 128.48, 128.3, 128.2, 101.2, 101.1, 75.8, 72.9, 72.7, 71.9, 71.7, 71.6, 70.9, 70.2, 62.7, 62.5, 51.4, 46.1, 34.0, 30.4, 29.3, 28.98, 28.97, 28.93, 25.7, 24.8; ESI HRMS calc'd for $C_{66}H_{66}O_{19}SNa$: 1217.3811 Found: 1217.3812. Anal. calc'd for $C_{66}H_{66}O_{19}S$: C 66.32, H 5.57. Found: C 66.01, H 5.47.

8-Methoxycarbonyloctyl (4-thio-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (110)

The thioester **109** (0.140 g; 0.12 mmol) was combined with dry methanol (10 mL) and argon was bubbled through the solution for twenty minutes before a small piece of sodium was added. After stirring overnight the reaction mixture was neutralized with acidic ion exchange resin, filtered and concentrated. The residue was taken up in water and poured onto a SepPak cartridge (5 g). The target **110** was eluted with a stepped gradient (10% steps) from 0 to 70% methanol in water (0.053 g; 1.0 mmol; 86%): $[\alpha]_D$ -5 (c 0.3, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD, δ) 4.37 (d, 1H, H-1', $J_{1',2'}$ = 7.7 Hz), 4.27 (d, 1H, H-1, $J_{1,2}$ = 7.9 Hz), 3.91-3.76 (m, 5H, OCH_2, H-6a', H-6a, H-6b, H-5'), 3.71-3.66 (m, 2H, H-6b', H-3'), 3.64 (s, 3H, CO_2CH_3), 3.57-3.52 (m, 3H, H-4, OCH_2, H-2'), 3.51 (dd, H-3, $J_{3,2}$ = $J_{3,4}$ = 8.9 Hz), 3.39 (ddd, 1H, H-5, J = 3.6 Hz, J = 4.2 Hz, J = 9.4 Hz), 3.33 (dd, 1H, H-4', $J_{4',5'}$ = 1.5 Hz, $J_{4',3'}$ = 4.6 Hz), 3.22 (dd, 1H, H-2), 2.31 (t, 2H,

$CH_2CO_2CH_3$, J = 7.4 Hz), 1.64-1.56 (m, 4H, 2xCH_2), 1.42-1.29 (m, 8H, 4xCH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.0, 105.8, 104.2, 81.1, 76.44, 76.41, 74.9, 74.0, 72.4, 70.9, 63.6, 62.0, 52.0, 45.4, 34.8, 30.7, 30.4, 30.3, 30.1, 27.0, 26.0; ESI HRMS calc`d for $C_{22}H_{40}O_{12}SNa$: 551.21327 Found: 551.21306.

Allyl (2,3-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (111)

Allyl (2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside[251] (2.340 g; 3.44 mmol) was suspended in 80% acetic acid (100 mL) before heating to 50 °C. After stirring overnight the reaction mixture was concentrated and the residue evaporated from toluene (2x20 mL) and recrystallized from ethyl acetate-hexanes to afford **111** (1.427 g; 2.41 mmol; 70%): $[\alpha]_D$ –10 (c 0.60, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.90-5.79 (m, 1H, H-b), 5.29-5.16 (m, 4H, H-c, H-d, H-3, H-2'), 4.93 (dd, 1H, H-2, $J_{2,1}$ = 8.0 Hz, $J_{2,3}$ = 9.2 Hz), 4.89 (dd, 1H, H-3', $J_{3',2'}$ = 10.2 Hz, $J_{3',4'}$ = 3.2 Hz), 4.54-4.49 (m, 3H, H-1, H-1', H-6a), 4.34-4.26 (m, 1H, H-a), 4.13-4.04 (m, 3H, H-4', H-a', H-6b), 3.95-3.88 (m, 1H, H-6a'), 3.87-3.80 (m, 2H, H-4, H-6b'), 3.66-3.60 (m, 1H, H-5), 2.78 (bs, 3H, O*H*), 2.48 (bs, 3H, O*H*), 2.11 (s, 3H, C(O)CH_3), 2.08 (s, 6H, 2 x C(O)CH_3), 2.04 (s, 6H, 2 x C(O)CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.55, 170.49, 170.1, 169.7, 169.4, 133.4,117.6, 101.0, 99.3, 76.3, 74.4, 73.5, 73.3, 72.6, 71.7, 70.0, 69.7, 67.9, 62.3, 62.2, 20.9, 20.84, 20.78, 20.68, 20.64; ESI HRMS calc`d for $C_{25}H_{36}O_{16}Na$: 615.1896 Found: 615.1895. Anal. calc`d for $C_{25}H_{36}O_{16}$: C 50.67, H 6.12. Found: C 50.48, H 6.33.

Allyl (2,3-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (112)

The diol **111** (0.700 g; 1.18 mmol) and 4-dimethylaminopyridine (5 mg) were dissolved in dichloromethane (10.0 mL) and pyridine (2.0 mL). The mixture was cooled to –78 °C and benzoyl chloride (0.151 mL; 1.3 mmol; 1.1 eq.) was added dropwise to the vigorously stirred solution. The mixture was allowed to warm to room temperature 3-4 hours. After stirring overnight benzoyl chloride (0.020 mL) was added and stirring was continued for a further 4 hours before methanol (1 mL) was added and the mixture was evaporated to dryness. Column chromatography on silica gel using hexanes-ethyl acetate-acetone (5:2:1) afforded **112** (0.461 g; 0.66 mmol; 56%): $[\alpha]_D$ 0 (c 0.75, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.10-8.03 (m, 2H, Ar), 7.64-7.57 (m, 1H, Ar), 7.54-7.46 (m, 2H, Ar), 5.83 (ddd, 1H, H-b), 5.27-5.16 (m, 4H, H-c, H-d, H-2', H-3), 4.96-4.91 (m, 2H, H-2, H-3'), 4.65 (dd, 1H, H-6a', $J_{6a',5'}$ = 6.4 Hz, $J_{6a',6b'}$ = 11.4 Hz), 4.51-4.41 (m, 4H, H-1, H-1', H-6a, H-6b'), 4.29 (dddd, 1H, H-a, J = 1.6 Hz, J = 1.8 Hz, J = 4.9 Hz, J = 13.2 Hz), 4.12-4.03 (m, 4H, H-3, H-6b, H-4', H-a'), 3.84 (dd, 1H, H-5', $J \sim J \sim$ 6.7 Hz), 3.78 (dd, 1H, H-4, $J \sim J \sim$ 9.6 Hz), 3.60 (ddd, 1H, H-5, J = 1.9 Hz, J = 5.0 Hz, J = 9.7 Hz), 2.087, 2.085, 2.079, 2.042, 2.039 (5s, 15H, 5 x C(O)CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 170.1, 170.0, 169.5, 169.3, 166.4, 133.5, 133.4, 129.7, 129.4, 128.7, 117.6, 101.1, 99.4, 76.3, 73.3, 72.8, 72.6, 72.3, 71.6, 70.0, 69.6, 66.9, 62.2, 62.0, 20.83, 20.80, 20.78, 20.69, 20.62; ESI HRMS calc`d for $C_{32}H_{40}O_{17}Na$: 719.2158 Found: 719.2158. Anal. calc`d for $C_{32}H_{40}O_{17}$: C 55.17, H 5.79. Found: C 54.80, H 5.99.

Allyl (2,3-di-*O*-acetyl-6-*O*-benzoyl-β-D-glucopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (113)

The lactoside **112** (0.150 g; 0.21 mmol) was dissolved in freshly distilled dichloromethane (5.0 mL) containing dry pyridine (175 μL; 2.11 mmol; 10 eq.). After cooling the solution to -78 °C, trifluoromethanesulfonic anhydride (106 μL; 0.63 mmol; 3.0 eq.) was added, and the reaction mixture was allowed to warm to 0°C over 3.5 hours. After this time, dichloromethane (25 mL) was added and the solution was washed with an equal volume of water, dried over sodium sulfate, and concentrated. The crude residue was taken up in *N,N*-dimethylformamide (5.0 mL) and tetrabutylammonium nitrite (0.187 g; 0.65 mmol; 3.0 eq.) was added. The mixture was stirred overnight at room temperature and then concentrated. Column chromatography of the yellow residue employing hexanes-ethyl acetate (1:1) as eluent yielded **113** (0.108 g; 0.16 mmol; 72%): $[\alpha]_D$ -23 (c 1.60, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07-8.03 (m, 2H, Ar), 7.64-7.59 (m, 1H, Ar), 7.53-7.47 (m, 2H, Ar), 5.88-5.78 (m, 1H, H-b), 5.28-5.22 (m, 1H, H-c), 5.22-5.14 (m, 2H, H-d, H-3), 5.01 (dd, 1H, H-3', $J_{3',2'} = J_{3',4'}$ = 9.1 Hz), 4.93 (dd, 1H, H-2, $J_{2,1}$ = 8.1 Hz, $J_{2,3}$ = 9.6 Hz), 4.88 (dd, 1H, H-2', $J_{2',1'}$ = 8.0 Hz), 4.75 (dd, 1H, H-6a, $J_{6a,6b}$ = 12.6 Hz, $J_{6a,5}$ = 4.0 Hz), 4.57-4.47 (m, 4H, H-6b, H-1', H-6a', H-1), 4.32-4.26 (m, 1H, H-a), 4.14-4.03 (m, 2H, H-a', H-6b'), 3.79 (dd, 1H, H-4, $J_{4,3} = J_{4,5}$ = 9.6 Hz), 3.66-3.55 (m, 3H, H-4', H-5, H-5'), 3.15 (bs, 1H, OH-4), 2.11, 2.05, 2.03, 2.02, 2.00, (5s, 15H, 5 OC(O)C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.2, 170.4, 169.8, 169.6, 169.3, 167.2, 133.7, 133.3, 129.8, 129.2, 128.7, 117.6, 101.0, 99.4, 76.7, 75.5, 74.5, 72.8, 72.6, 71.6, 71.5, 70.0, 68.5, 63.1, 61.9, 60.4, 20.83, 20.75, 20.69, 20.66, 20.58; ESI HRMS calc`d for $C_{32}H_{40}O_{17}Na$: 719.2158 Found: 719.2161. Anal. calc`d for $C_{32}H_{40}O_{17}$: C 55.17, H 5.79. Found: C 55.00, H 5.91.

Allyl (2,3-di-*O*-acetyl-4-*S*-acetyl-6-*O*-benzoyl-4-thio-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (114)

The alcohol **113** (0.260 g; 0.37 mmol) was dissolved in dichloromethane (5.0 mL) and pyridine (0.30 mL; 3.71 mmol; 10.0 eq.). The flask was flushed with argon, and the solution was cooled to –78 °C before addition of trifluoromethanesulfonic anhydride (0.190 mL; 11.13 mmol; 3.0 eq.). The mixture was allowed to warm to –20 °C over a period of 2 hours, was stirred at room temperature for 15 minutes, and was then diluted with dichloromethane (50 mL) and washed with an equal volume of water. The organic layers were dried over sodium sulfate, filtered and concentrated. Potassium thioacetate (0.130 g; 1.14 mmol; 3.0 eq.) and *N*,*N*-dimethylformamide (5.0 mL) were added to the residue and the mixture was stirred at room temperature for 1.5 hours. The brown solution was diluted with ethyl acetate (60 mL), washed once with an equal volume of water, dried over sodium sulfate, filtered, and concentrated. Chromatography of the residue on a silica gel column (20 cm x 2 cm) containing a 1.5 cm plug of activated carbon with hexanes-ethyl acetate (2:1) as eluent afforded **114** (0.228 g; 0.30 mmol; 81%): $[\alpha]_D$ –28.7 (c 1.05, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.05-8.02 (m, 2H, Ar), 7.62-7.58 (m, 1H, Ar), 7.53-7.48 (m, 2H, Ar), 5.82 (dddd, 1H, H-b, J = 5.0 Hz, J = 6.1 Hz, J = 10.5 Hz, J = 17.1 Hz), 5.25 (dddd, 1H, H-c, J = 1.7 Hz, J = 1.7 Hz, J = 1.7 Hz, J = 17.1 Hz), 5.21-5.15 (m, 3H, H-d, H-3, H-3'), 4.94 (dd, 1H, H-2', $J_{2',1'}$ = 7.8 Hz, $J_{2',3'}$ = 10.2 Hz), 4.91 (dd, 1H, H-2, $J_{2,1}$ = 7.9 Hz, $J_{2,3}$ = 9.6 Hz), 4.51-4.40 (m, 5H, H-1, H-6a, H-6a', H-1', H-6b'), 4.37 (dd, 1H, H-4', J = 1.6 Hz, J = 4.5 Hz), 4.28 (dddd, 1H, H-a, J = 1.5 Hz, J = 1.5 Hz, J = 4.9 Hz, J = 13.2 Hz), 4.16-4.12 (m, 1H, H-5'), 4.09-4.02 (m, 2H, H-6b, H-a'), 3.75 (dd, 1H, H-4, J = J = 9.6 Hz), 3.57 (ddd, 1H, H-5, J = 2.1 Hz, J = 5.0 Hz, J = 10.0 Hz), 2.39 (s, 3H, SC(O)CH_3), 2.09, 2.08, 2.05, 2.04, 1.95 (5s, 15H, 5

OC(O)CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 193.2, 170.3, 169.9, 169.8, 169.5, 168.9, 166.0, 133.5, 133.3, 129.7, 129.3, 128.6, 117.6, 101.5, 99.5, 76.3, 72.7, 72.3, 71.50, 71.46, 71.3, 70.4, 70.0, 63.0, 61.9, 45.9, 30.7, 20.82, 20.79, 20.68, 20.57 (2C); ESI HRMS calc`d for $C_{34}H_{42}O_{17}SNa$: 777.2035. Found: 777.2035. Anal. calc`d for $C_{34}H_{42}O_{17}S$: C 54.11, H 5.61. Found: C 54.30, H 5.99.

8-Methoxycarbonyloctyl 3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (115)

The galactoside[281] (0.893 g; 2.55 mmol) was concentrated from toluene (2 x 10 mL), combined with dibutyltin oxide (0.634 g; 2.55 mmol; 1.0 eq.) and dry toluene (25 mL) was added. The suspension was fitted with a Dean-Stark trap and heated at reflux. After several hours, the remaining 10 mL of the solution was cooled to room temperature and combined with tetrabutylammonium iodide (0.941 g; 2.55 mmol; 1.0 eq.) and *p*-methoxybenzyl chloride (0.450 mL; 3.25 mmol; 1.3 eq.). This mixture was heated at 85 °C for 30 minutes and then concentrated. Chromatography of the residue on silica gel afforded the target **115** (0.503 g; 1.07 mmol; 42%): $[\alpha]_D$ –7 (c 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32-7.28 (m, 2H, Ar), 6.91-6.87 (m, 2H, Ar), 4.68 (s, 2H, OCH_2Ar), 4.24 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 3.99-3.94 (m, 2H, H-4, H-6a), 3.90 (ddd, 1H, OCH_2H$_2$, J = 6.3 Hz, 6.4 Hz, 8.7 Hz), 3.84-3.78 (m, 1H, H-6b), 3.81 (s, 3H, OCH_3), 3.78-3.73 (m, 1H, H-2), 3.66 (s, 3H, OCH_3), 3.54-3.47 (m, 2H, OCH_2CH$_2$, H-5), 3.42 (dd, 1H, H-3, $J_{3,2}$ = 9.4 Hz, $J_{3,4}$ = 3.5 Hz), 2.58 (bs, 1H, OH-4), 2.38 (d, 1H, OH-2, $J_{OH\text{-}2,H\text{-}2}$ = 2.1 Hz), 2.30 (dd, 2H, CH_2CO$_2$Me, J = J = 7.6 Hz), 2.17 (dd, 1H, J 4.0 = Hz, J 8.4 = Hz), 1.66-1.56 (m, 4H, 2 x CH_2), 1.38-1.27 (m, 8H, 4 x CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3, 159.5, 129.7, 129.5, 114.0, 103.1, 79.8, 74.3, 71.9, 71.1, 70.0, 67.2, 62.6, 55.3, 51.4, 34.1, 29.6, 29.11, 29.07, 28.99, 25.8, 24.9; ESI HRMS calc'd for

$C_{24}H_{38}O_9Na$: 493.2408. Found: 493.2404. Anal. calc'd for $C_{24}H_{38}O_9$: C 61.26, H 8.14. Found: C 61.02, H 8.13.

8-Methoxycarbonyloctyl 4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (116)

The triol **115** (0.700 g; 1.49 mmol) was dissolved in DMF (10 mL) and benzaldehyde dimethylacetal (0.45 mL; 2.0 eq.). A catalytic amount of *p*-toluenesulfonic acid monohydrate was added, and the mixture was stirred overnight, then made basic with triethylamine. Following concentration, the residue was chromatographed on silica gel using hexanes-ethyl acetate (3:2) as eluent to afford **116** (0.679 g; 1.22 mmol; 82%): $[\alpha]_D$ 27 (c 1.15, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.54-7.50 (m, 2H, Ar), 7.37-7.29 (m, 5H, Ar), 6.87-6.84 (m, 2H, Ar), 5.45 (s, 1H, C*H*Ph), 4.71 (d, 1H, CH_2Ar, J = 12.0 Hz), 4.67 (d, 1H, CH_2Ar), 4.31-4.25 (m, 2H, H-1, H-6a), 4.10 (d, 1H, $J_{4,3}$ = 3.5 Hz), 4.05-3.90 (m, 3H, H-6b, H-2, OCH_2CH_2), 3.79 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 3.54-3.44 (m, 2H, H-3, OCH_2CH_2), 3.33 (bs, 1H, H-5), 2.49 (bs, 1H, OH-2), 2.29 (dd, 2H, J = 7.5 Hz), 1.68-1.57 (m, 4H), 1.39-1.25 (m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3, 159.3, 137.8, 130.2, 129.4, 128.9, 128.1, 126.4, 113.8, 102.9, 101.1, 78.8, 73.3, 71.1, 70.0, 69.7, 69.3, 66.7, 55.3, 51.4, 34.1, 29.5, 29.2, 29.1, 29.0, 25.9, 24.9; HRMS calc'd for $C_{31}H_{42}O_9Na$: 581.2721. Found: 581.2720. Anal. calc'd for $C_{31}H_{42}O_9$: C 66.65, H 7.58. Found: C 66.23, H 7.62.

8-Methoxycarbonyloctyl (2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (117)

Method A: 2,3,4-Tri-*O*-benzyl-α/β-L-fucopyranose (0.467 g; 1.08 mmol), triphenylphosphine (0.850 g; 3.24 mmol; 3.0 eq.) and carbon tetrabromide (0.1.071 g;

3.23 mmol; 3.0 eq.) were combined, and *N,N*-dimethylformamide (15 mL) was added. The mixture was stirred for four hours at room temperature, before **116** was added as a solid (0.401 g; 0.71 mmol; 0.66 eq.). After 36-40 hours the reaction mixture was diluted with ethyl acetate (100 mL), washed with water (100 mL), dried over sodium sulfate, filtered and concentrated. After subjecting the residue to reduced pressure to remove traces of *N,N*-dimethylformamide, column chromatography on silica gel using hexanes-ethyl acetate (3:1) as eluent afforded the β-fucoside (0.052 g; 0.05 mmol; 7%) and **117** (0.578 g; 0.65 mmol; 83%).

Method B: The acceptor **116** (0.305 g; 0.55 mmol), powdered 3Å molecular sieves, and 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide[282] (2.0 eq.) were combined under an argon atmosphere in dichloromethane/*N,N*-dimethylformamide (8 mL) and stirred for thirty minutes. After this time, tetrabutylammonium bromide (0.233 g; 2.0 eq.) was added, and the mixture was stirred for 72 hours at room temperature, then filtered and diluted with dichloromethane (60 mL). The solution was washed with water (2 x 50 mL), dried over sodium sulfate, filtered and concentrated. Column chromatography on silica gel with hexanes/ethyl acetate (4/1 – 3/1) yielded the β-fucoside (0.030 g; 0.03 mmol; 6%) and **117** (0.420 g; 0.44 mmol; 79 %): $[\alpha]_D$ – 28 (c 0.55, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.55-7.51 (m, 2H, Ar), 7.39-7.17 (m, 20H, Ar), 6.79-6.75 (m, 2H, Ar), 5.62 (d, 1H, H-1', $J_{1',2'}$ = 3.7 Hz), 5.41 (s, 1H, C*H*Ph), 4.94 (d, 1H, CH_2Ar, J = 11.7 Hz), 4.86 (d, 1H, CH_2Ar, J = 11.6 Hz), 4.73 (d, 1H, CH_2Ar, J = 11.6 Hz), 4.68 (d, 1H, CH_2Ar, J = 12.0 Hz), 4.66 (d, 1H, CH_2Ar, J = 11.5 Hz), 4.63 (d, 1H, CH_2Ar, J = 11.7 Hz), 4.56 (d, 1H, CH_2Ar, J = 12.0 Hz), 4.50 (d, 1H, CH_2Ar, J = 11.4 Hz), 4.45 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 4.42 (q, 1H, H-5', $J_{5',6'}$ = 6.5 Hz), 4.30 (dd, 1H, H-6a, $J_{6a,5}$ = 1.2 Hz,

$J_{6a,6b}$ = 12.2 Hz), 4.16 (dd, 1H, H-2, $J_{2,3}$ = 9.4 Hz), 4.12 (d, 1H, H-4, $J_{4,3}$ = 3.8 Hz), 4.04-3.99 (m, 2H, H-6b, H-2'), 3.96 (dd, 1H, H-3', $J_{3,2}$ = 10.2 Hz, $J_{3,4}$ = 2.8 Hz), 3.90 (ddd, 1H, OCH_2CH$_2$, J = 9.2 Hz, J_{vic} = 6.8 Hz, J_{vic} = 6.8 Hz), 3.79 (dd, 1H, H-3), 3.76 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3), 3.65 (m, 1H, H-4'), 3.39 (ddd, 1H, OCH_2CH$_2$, J = 9.2 Hz, J_{vic} = 6.9 Hz, J_{vic} = 6.9 Hz), 3.33 (bs, 1H, H-5), 2.30 (dd, 2H, $J \sim J \sim 7$ Hz), 1.64-1.49 (m, 4H), 1.32-1.22 (m, 8H), 1.11 (d, 3H, H-6'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.2, 159.0, 139.1, 138.9, 138.6, 137.8, 130.5, 128.7, 128.6, 128.3, 128.14, 128.085, 128.078, 128.04, 127.8, 127.35, 127.31, 127.30, 127.29, 126.3, 113.7, 101.8, 100.9, 97.0 ($^1J_{C1',H1'}$ = 173.5 Hz), 81.4, 79.6, 78.2, 76.0, 74.7, 73.0, 72.9, 72.5, 71.6, 70.3, 69.4, 69.3, 66.4, 66.2, 55.3, 51.4, 34.1, 29.7, 29.33, 29.26, 29.1, 26.1, 24.9, 16.6; HRMS calc'd for $C_{58}H_{70}O_{13}Na$: 997.4709. Found: 997.4709. Anal. calc'd for $C_{58}H_{70}O_{13}$: C 71.44, H 7.24. Found: C 71.78, H 7.43. Selected NMR data for the β isomer: ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.48 (s, 1H, C*H*Ph), 4.92 (d, 1H, H-1', $J_{1',2'}$ = 7.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 103.6 , 102.4 ($^1J_{C1',H1'}$ = 162.8 Hz).

8-Methoxycarbonyloctyl (2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-4,6-*O*-benzylidene-β-D-galactopyranoside (118)

The disaccharide **117** (0.400 g; 0.41 mmol) was dissolved in 95% aqueous acetonitrile (20 mL). Ceric ammonium nitrate (0.900 g; 1.64 mmol; 4.0 eq.) was added, and the reaction mixture was stirred at room temperature for 35 minutes, then diluted with dichloromethane (150 mL), washed with water (2 x 75 mL), dried over sodium sulfate, filtered and concentrated. The residue was chromatographed on silica gel using hexanes-ethyl acetate (5:3) as eluent to afford the title compound **118** (0.324 g; 0.38 mmol; 93%): $[\alpha]_D$ – 51 (c 0.60, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55-7.52 (m, 2H, Ar), 7.42-

7.21 (m, 18H, Ar), 5.56 (s, 1H, C*H*Ph), 5.28 (d, 1H, H-1', $J_{1',2'}$ = 3.6 Hz), 4.98 (d, 1H, OCH_2Ar, J = 11.6 Hz), 4.83 (d, 1H, CH_2Ar, J = 11.7 Hz), 4.82, (d, 1H, CH_2Ar, J = 11.5 Hz), 4.78 (d, 1H, CH_2Ar, J = 11.5 Hz), 4.74 (d, 1H, CH_2Ar, J = 11.7 Hz), 4.66 (d, 1H, OCH_2Ar, J = 11.6 Hz), 4.36 (m, 1H, H-1), 4.31 (dd, 1H, H-6a, $J_{6a,5}$ = 1.4 Hz, $J_{6a,6b}$ = 12.3 Hz), 4.22-4.17 (m, 2H, H-4, H-5'), 4.10-4.05 (m, 2H, H-6b, H-2'), 3.98 (dd, 1H, H-3', $J_{3',2'}$ = 10.1 Hz $J_{3',4'}$ = 2.1 Hz), 3.88 (ddd, 1H, OCH_2CH$_2$, J = 9.3 Hz, J_{vic} = 7.1 Hz, J_{vic} = 7.1 Hz), 3.87-3.84 (m, 2H, H-2, H-3), 3.71-3.67 (m, 2H, OH-3, H-4'), 3.66 (s, 3H, OCH_3), 3.45 (ddd, 1H, OCH_2CH$_2$, J = 9.3 Hz, J_{vic} = 7.0 Hz, J_{vic} = 7.0 Hz), 3.42 (bs, 1H, H-5), 2.29 (dd, 2H, J = 7.6 Hz), 1.66-1.54 (m, 4H), 1.35-1.24 (m, 8H), 1.13 (d, 3H, H-6', $J_{6',5'}$ = 6.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.2, 138.8, 138.7, 137.9, 137.7, 129.1, 128.39, 128.37, 128.36, 128.25, 128.16, 128.13, 127.8, 127.50, 127.47, 127.35, 126.6, 101.8, 101.4, 99.2, 79.8, 77.8, 77.6, 76.7, 75.6, 74.8, 73.69, 73.67, 72.95, 69.5, 69.3, 66.8, 66.6, 51.4, 34.1, 29.7, 29.3, 29.2, 29.1, 26.0, 24.9, 16.7; ESI HRMS calc'd for $C_{50}H_{62}O_{12}Na$: 854.4134. Found: 854.4132. Anal. calc'd for $C_{50}H_{62}O_{12}$: C 70.24, H 7.31. Found: C 70.31 H 7.33.

8-Methoxycarbonyloctyl (2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-4,6-*O*-benzylidene-β-D-gulopyranoside (119)

The equatorial alcohol **118** (0.130 g; 0.13 mmol) was dissolved in freshly distilled dichloromethane (5.0 mL) and pyridine (0.065 mL; 0.80mmol; 6.0 eq.), then cooled to -20 °C. Trifluoromethanesulfonic anhydride (0.045 mL; 0.27 mmol; 2.0 eq.) was added, and the mixture was allowed to warm to –10 °C over a period of 45 minutes. The reaction mixture was diluted with dichloromethane (50 mL), washed with water (1 x 50 mL), dried over sodium sulfate, filtered and concentrated. The residue was dissolved in

acetonitrile (5.0 mL) containing several drops of water and tetrabutylammonium nitrite (0.193 g; 0.67 mmol; 5.0 eq.) was added. The mixture was stirred at 40 °C for 12 hours, then concentrated. Chromatography of the mixture on silica gel with hexanes:ethyl acetate (3/1-5/2) as eluent yielded **119** (0.077 g; 0.09 mmol; 59 %): $[\alpha]_D$ –14 (c 0.27, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53-7.49 (m, 2H, Ar), 7.42-7.27 (m, 18H, Ar), 5.54 (s, 1H, C*H*Ph), 4.97 (d, 1H, CH_2Ar), 4.88 (d, 1H, H-1', $J_{1',2'}$ = 3.7 Hz), 4.85 (d, 1H, CH_2Ar), 4.80 (d, 1H, CH_2Ar, J = 11.7 Hz), 4.77 (d, 1H CH_2Ar, J = 11.7 Hz), 4.70 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 4.69 (d, 1H, CH_2Ar), 4.67 (d, 1H, CH_2Ar), 4.32 (dd, 1H, H-6a, $J_{6a,5}$ = 1.3 Hz, $J_{6a,6b}$ = 12.4 Hz), 4.22 (q, 1H, H-5', $J_{5',6'}$ = 7.0 Hz), 4.13-4.10 (m, 2H, H-3, H-4), 4.07-4.03 (m, 2H, H-6b, H-2'), 3.99 (dd, 1H, H-3', $J_{3',2'}$ = 10.0 Hz, $J_{3',2'}$ = 2.8 Hz), 3.90 (ddd, 1H, OCH_2CH$_2$, J_{vic} = 6.6 Hz, J_{vic} = 6.6 Hz, J = 9.3 Hz), 3.84 (dd, 1H, H-2, $J_{2,3}$ = 2.8 Hz), 3.75 (bs, 1H, H-5), 3.70-3.68 (m, 1H, H-4'), 3.67 (s, 3H, OCH_3), 3.60 (bs, 1H, O*H*-3), 3.45 (ddd, 1H, OCH_2CH$_2$, J = 6.9 Hz, J = 6.9 Hz, J = 9.3 Hz), 2.30 (dd, 2H, J = 7.6 Hz), 1.64-1.53 (m, 4H), 1.37-1.24 (m, 8H), 1.13 (d, 3H, H-6'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.2, 138.60, 138.59, 137.9, 137.8, 129.1, 128.5, 128.4, 128.3, 128.21 (2C), 128.20, 128.0, 127.59, 127.57, 127.3, 126.4, 101.5, 98.7, 94.8, 80.0, 77.5, 75.8, 75.7, 74.8, 74.4, 72.71, 72.67, 69.4, 68.7, 67.3, 66.9, 65.6, 51.4, 34.1, 29.8, 29.32, 29.27, 29.1, 26.1, 24.9, 16.6; ESI HRMS calc'd for $C_{50}H_{62}O_{12}Na$: 877. 4134. Found: 877.4132. Anal. calc'd for $C_{50}H_{62}O_{12}$: C 70.24, H 7.31. Found: C 70.02 H 7.32.

8-Methoxycarbonyloctyl (2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-2-*S*-acetyl-4,6-*O*-benzylidene-3-thio-β-D-galactopyranoside (120)

The alcohol **119** (0.060 g; 70 μmol) was dissolved in freshly distilled dichloromethane (4 mL) and pyridine (0.030 mL; 0.37 mmol; 6 eq.), then cooled to –20 °C.

Trifluoromethanesulfonic anhydride (0.040 mL; 0.24 mmol; 3 eq.) was added and the reaction mixture was allowed to warm to 0 °C for 1 hour, then to room temperature. After 8 hours, the mixture was diluted with dichloromethane (15 mL), washed with water, dried over sodium sulfate, filtered, and concentrated. The residue was combined with potassium thioacetate (0.048 g; 0.42 mmol; 6.0 eq.) and degassed *N,N*-dimethylformamide. After stirring overnight, potassium thioacetate (0.048 g; 0.42 mmol; 6.0 eq.) was added and the temperature was increased to 60 °C. After 6 hours the mixture was diluted with ethyl acetate (25 mL), washed with water (2 x 15 mL), dried over sodium sulfate, filtered, and concentrated. Chromatgraphy of the residue on silica gel using hexanes-ethyl acetate (7:2) as eluent yielded **120** (0.041 g; 45 μmol; 64%): $[\alpha]_D$ 5 (c 0.43, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.52-7.49 (m, 2H, Ar), 7.38-7.23 (m, Ar, 18 H), 5.50 (s, 1H, C*H*Ph), 5.37 (d, 1H, H-1', $J_{1,2}$ = 3.6 Hz), 4.96 (d, 1H, CH_2Ph, *J* = 11.7 Hz), 4.81 (d, 1H, CH_2Ph, *J* = 11.7 Hz), 4.77 (d, 1H, CH_2Ph, *J* = 11.7 Hz), 4.69 (m, 2H, 2 x CH_2Ph), 4.64 (d, 1H, CH_2Ph, *J* = 11.7 Hz), 4.51 (d, 1H, H-1, $J_{1,2}$ = 7.4 Hz), 4.32-4.28 (m, 2H, H-6a, H-5'), 4.09 (dd, 1H, H-3, $J_{3,4}$ = 3.4 Hz, $J_{3,2}$ = 10.9 Hz), 4.06-4.02 (m, 2H, H-6b, H-2'), 4.00 (dd, 1H, H-4, $J_{4,5}$ = 0.6 Hz), 3.93 (dd, 1H, H-2), 3.92-3.86 (m, 2H, H-3', OCH_2), 3.66 (s, 3H, CO_2CH_3), 3.64-3.62 (m, 1H, H-4'), 3.59-3.57 (m, 1H, H-5), 3.44 (ddd, 1H, OCH_2, *J* = 7.1 Hz, *J* = 7.1 Hz, *J* = 9.0 Hz), 2.30-2.27 (m, 2H, CH_2CO_2Me), 2.09 (s, 3H, SC(O)CH_3), 1.63-1.55 (m, 4H), 1.32-1.24 (m, 8H), 1.10 (d, 3H, H-6'); ^{13}C NMR (150 MHz, $CDCl_3$, assigned from HMQC and HMBC) δ 195.1 (S*C*(O)CH_3), 174.3 (CO_2Me), 101.1 (*C*HPh), 103.8 (C1), 97.7 (C-1', $^1J_{C1',H1'}$ = 169.6 Hz), 79.4 (C-3'), 78.2 (C-4'), 76.8 (C-2'), 76.0 (C-4), 74.7, 73.06, 73.04, 73.01 (4x*C*H_2Ph), 72.2 (C-2), 69.9 (OCH_2), 69.1 (C-6), 68.2 (C-5), 66.8 (C-5'), 51.4 (CO_2CH_3), 48.9 (C-3),

16.9 (C-6'); ESI HRMS calc'd for $C_{52}H_{64}O_{12}S$ Na: 935.4011. Found: 935.4009. Anal. calc'd for $C_{52}H_{64}O_{12}S$: C 68.60, H 7.06. Found: C 68.89 H 7.21.

2-(Trimethylsilyl)ethyl 4,6-*O*-benzylidene-3-*S*-benzyl-3-thio-β-D-galactopyranoside (121)

The thioester **18** (0.0559 g; 0.11 mmol) was dissolved in *N,N*-dimethylformamide and argon was bubbled through the solution for 30 minutes before adding hydrazine acetate (0.0194 g; 0.21 mmol; 2.0 eq.). After 3 hours the solution was diluted with ethyl acetate (20 mL), washed with water (2 x 10 mL), dried over sodium sulfate, filtered and concentrated. The residue was dissolved in toluene (10 mL) and concentrated, then dissolved in degassed *N,N*-dimethylformamide (3 mL), and benzyl bromide (0.019 mL; 0.15 mmol; 1.5 eq.) before adding 1,8-diazabicyclo-[5.4.0]-undec-7-ene (0.020 ml). After stirring overnight the reaction mixture was diluted with ethyl acetate (15 mL), washed with 1M HCl (10 mL), saturated sodium bicarbonate solution (10 mL), and water (10 mL), then dried over sodium sulfate, filtered and concentrated. ^{1}H NMR data was recorded for the crude product, 2-(trimethylsilyl)ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*S*-benzyl-3-thio-β-D-galactopyranoside: (600 MHz, $CDCl_3$) δ 8.10-8.07 (m, 2H, Ar), 7.59-7.56 (m, 1H, Ar), 7.55-7.52 (m, 2H, Ar), 7.48-7.45 (m, 2H, Ar), 7.39-7.30 (m, 4H, Ar), 7.22-7.16 (m, 3H, Ar), 5.57 (dd, 1H, H-2, $J_{2,1}$ = 7.8 Hz, $J_{2,3}$ = 11.1 Hz), 5.52 (s, 1H, C*H*Ph), 4.60 (d, 1H, H-1), 4.30 (dd, 1H, H-6a, $J_{6a,5}$ = 1.3 Hz, $J_{6a,6b}$ = 12.3 Hz), 4.07 (d, 1H, H-4, $J_{4,3}$ = 3.3 Hz), 4.03 (dd, 1H, H-6b, $J_{6b,5}$ = 1.8 Hz), 3.99 (dd, 1H, H-a, *J* = 5.4 Hz, *J* = 9.7 Hz, *J* = 10.8 Hz), 3.82 (d, 1H, SCH_2, *J* = 12.8 Hz), 3.78 (d, 1H, SCH_2), 3.52 (ddd, 1H, H-a', *J* = 6.6 Hz, *J* = 9.7 Hz, *J* = 10.4 Hz), 3.45 (s, 1H, H-5), 2.99 (dd, 1H, H-3), 0.88 (ddd, 1H, H-b, *J* = 6.6 Hz, *J* = 10.8 Hz, *J* = 13.9 Hz), 0.81 (ddd, 1H, H-b', *J* =

5.4 Hz, J = 10.5 Hz, J = 13.9 Hz), -0.1 (s, 9H, $Si(CH_3)_3$); ESI HRMS calc'd for $C_{32}H_{38}O_6SSiNa$: 601.2051. Found: 601.2051. This crude material was combined with a small piece of sodium in methanol and stirred overnight at 55 °C, then neutralized by addition of acidic ion exchange resin, filtered and concentrated. Chromatography on silica gel using hexanes-ethyl acetate (6:1) as eluent afforded **121** (0.046 mg ; 0.079 mmol; 71%): $[\alpha]_D$ -16 (c 0.69, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 7.52-7.49 (m, 2H, Ar), 7.39-7.29 (m, 8H, Ar), 5.48 (s, 1H, C*H*Ph), 4.31-4.27 (m, 2H, H-1, H-6a), 4.06 (ddd, 1H, H-a, J = 5.7 Hz, J = 9.5 Hz, J = 11.5 Hz), 4.04-3.94 (m, 2H, SCH_2Ar), 4.00 (dd, 1H, H-6b, $J_{6b,5}$ = 1.8 Hz, $J_{6a,6b}$ = 12.4 Hz), 3.97 (bd, 1H, H-4, J = 3.3 Hz), 3.93 (dd, 1H, H-2, $J_{2,1}$ = 7.7 Hz, $J_{2,3}$ = 10.8 Hz), 3.58 (ddd, 1H, H-a', J = 6.1 Hz, J = 9.5 Hz, J = 11.5 Hz), 3.40 (s, 1H, H-5), 2.76 (m, 1H, H-3), 2.50 (bs, 1H, OH), 1.03 (m, 2H, H-b, H-b'), 0.03 (s, 9H, Si(CH3)3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.6, 137.7, 129.1, 128.7, 128.5, 128.0, 127.0, 126.1, 103.8, 101.1, ~ 77, 71.4, 69.1, 68.7, 67.0, 49.4, 36.6, 18.1, -1.4; ESI HRMS calc'd for $C_{25}H_{34}O_5SSiNa$: 497.1789. Found: 497.1790. Anal. calc'd for $C_{25}H_{34}O_5SSi$: C 63.26, H 7.22. Found: C 63.00, H 7.41.

2-(Trimethylsilyl)ethyl (2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-2-*S*-benzyl-4,6-*O*-benzylidene-3-thio-β-D-galactopyranoside (122)

The acceptor **121** (0.030 g; 63 μmol), crushed 3Å molecular sieves, and tetrabutylammonium bromide (0.410 g; 0.13 mmol) were stirred for 2 hours in dichloromethane (1 mL) and *N,N*-dimethylformamide (1 mL). Freshly prepared 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide[282] (0.070 g; 0.14 mmol; 2.2 eq.) in the same solvents (3.0 mL) was added to this mixture via syringe. After stirring for two days the mixture was filtered through a pad of Celite and diluted with ethyl acetate (20 mL). This

solution was washed with water (2 x 10 mL), dried over sodium sulfate, filtered and concentrated. Chromatography on silica gel using hexanes and ethyl acetate (6:1) as eluent afforded the disaccharide **122** (0.0415 g; 47 μmol; 74%): $[\alpha]_D$ –45 (c 0.51, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.52-7.48 (m, 2H, Ar), 7.37-7.25 (m, 15H, Ar), 7.20-7.15 (m, 8H, Ar), 5.75 (d, 1H, H-1', $J_{1',2'}$ = 3.7 Hz), 5.40 (s, 1H, C*H*Ph), 4.97 (d, 1H, CH_2Ar, J = 11.6 Hz), 4.86 (d, 1H, CH_2Ar, J = 11.8 Hz), 4.85 (d, 1H, CH_2Ar, J = 11.8 Hz), 4.78 (d, 1H, CH_2Ar, J = 11.6 Hz), 4.72 (d, 1H, CH_2Ar, J = 11.8 Hz), 4.66 (d, 1H, CH_2Ar, J = 11.8 Hz), 4.41 (ddd, 1H, H-5', $J_{5',6'}$ = 6.6 Hz), 4.37 (d, 1H, H-1, $J_{1,2}$ = 7.5 Hz), 4.22 (dd, 1H, H-6a, $J_{6a,5}$ = 1.1 Hz, $J_{6a,6b}$ = 12.1 Hz), 4.12 (dd, 1H, H-2', $J_{2',3'}$ = 10.2 Hz), 4.04 (dd, 1H, H-2, $J_{2,3}$ = 10.4 Hz), 3.99 (dd, 1H, H-3', $J_{3',4'}$ = 2.7 Hz), 3.97-3.90 (m, 2H, H-6b, H-a), 3.89 (d, 1H, SCH_2, J = 13.0 Hz), 3.86 (d, 1H, SCH_2), 3.79 (bd, 1H, H-4, $J_{4,3}$ = 3.3 Hz), 3.66 (s, 1H, H-4'), 3.43 (m, 1H, H-a' 3.30 (s, 1H, H-5), 2.85 (dd, 1H, H-3), 1.11 (d, 3H, 3 x H-6), 0.96-0.85 (m, 2H, H-b, H-b'), -0.03 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.0, 138.9, 138.51, 138.47, 137.7, 129.0, 128.7, 128.32, 128.26, 128.20 (2C), 128.13, 128.11, 128.00, 127.41, 127.38, 127.34, 127.30, 126.8, 126.2, 103.2, 101.2, 97.7, 79.5, 78.2, 78.0, 76.5, 74.7, 73.5, 73.2, 73.0, 69.0, 68.3, 66.8, 66.6, 50.5, 36.6, 18.2, 16.8, -1.5; ESI HRMS calc'd for $C_{52}H_{62}O_9SSiNa$: 913.3782. Found: 913.3787. Anal. calc'd for $C_{52}H_{62}O_9SSi$: C 70.08, H 7.01. Found: C 69.99, H 7.17.

Allyl 4,6-*O*-benzylidene-3-*S*-thioethyl-3-thio-β-D-galactopyranoside (123)

The thiol **29** (0.108 g; 0.25 mmol) was combined with diethyl-*N*-(ethylsulfanyl) hydrazodicarboxylate[283] (0.119 g; 0.50 mmol; 2.0 eq.) in dichloromethane (5.0 mL). After stirring for 8 hours, the mixture was diluted with dichloromethane (15 mL), washed with equal volumes of 1M HCl, saturated sodium bicarbonate solution and water, then

dried over sodium sulfate, filtered and concentrated. ^{1}H NMR and mass spectra were recorded for the crude product, allyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*S*-thioethyl-3-thio-β-D-galactopyranoside: (600 MHz, $CDCl_3$) δ 8.09-8.06 (m, 2H, Ar), 7.57-7.53 (m, 3H, Ar), 7.46-7.42 (m, 2H, Ar), 7.38-7.33 (m, 3H, Ar), 5.76 (dddd, 1H, H-b, J = 4.8 Hz, J = 6.2 Hz, J = 10.6 Hz, J = 17.3 Hz), 5.59 (s, 1H, C*H*Ph), 5.45 (dd, 1H, H-2, $J_{2,1}$ = 7.8 Hz, $J_{2,3}$ = 11.6 Hz), 5.19 (dddd, 1H, H-c, J = 1.6 Hz, J =1.6 Hz, J = 1.6 Hz, J = 17.2 Hz), 5.07 (dddd, 1H, H-d, J = 1.4 Hz, J =1.4 Hz, J = 1.4 Hz, J = 10.4 Hz), 4.70 (d, 1H, H-1), 4.37-4.32 (m, 3H, H-6a, H-4, H-a), 4.14-4.09 (m, 2H, H-6b, H-a'), 3.59-3.57 (m, 1H, H-5), 3.28 (dd, 1H, H-3, $J_{3,4}$ = 3.3 Hz), 2.59-2.50 (m, 2H, SC*H*$_2$CH$_3$), 1.08 (dd, 3H, SCH$_2$C*H*$_3$, J = J = 7.3 Hz); ESI HRMS calc'd for $C_{25}H_{28}O_6S_2Na$: 511.1220. Found: 511.1224. The residue was combined with a small piece of sodium in methanol/dichloromethane (1:1; 4 mL) and stirred overnight. After neutralization of the solution with acidic ion exchange resin, filtration, and concentration, the residue was chromatographed on silica gel using hexanes/ethyl acetate (7:2) as eluent to yield **123** (0.086 g; 0.22 mmol 89%): $[\alpha]_D$ –159 (c 0.98, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.54-7.51 (m, 2H, Ar), 7.38-7.33 (m, 3H, Ar), 5.96 (dddd, 1H, H-b, J = 6.4 Hz, J = 5.3 Hz, J = 10.4 Hz, J = 17.2 Hz), 5.56 (s, 1H, C*H*Ph), 5.33 (dddd, 1H, H-c, J = 1.6 Hz, J = 1.6 Hz, J = 1.6 Hz, J = 17.2 Hz), 5.23 (dddd, 1H, H-d, J = 1.3 Hz, J = 1.3 Hz, J = 1.3 Hz, J = 10.4 Hz), 4.44 (dddd, 1H, H-a, J = 1.4 Hz, J = 1.4 Hz, J = 5.3 Hz, J = 12.6 Hz), 4.40 (d, 1H, H-1, $J_{1,2}$ = 7.5 Hz), 4.34 (dd, 1H, $J_{6a,5}$ = 1.5 Hz, $J_{6a,6b}$ = 12.4 Hz), 4.28 (dd, 1H, H-4, $J_{4,3}$ = 3.4 Hz, $J_{4,5}$ = 0.9 Hz), 4.14 (dddd, 1H, H-a', J =1.3 Hz, 1.3 Hz, 6.4 Hz, 12.5 Hz), 4.10 (dd, 1H, H-6b, $J_{6b,5}$ = 1.9 Hz), 3.88 (dd, 1H, H-2, $J_{2,3}$ = 11.1 Hz), 3.52-3.50 (m, 1H, H-5), 3.02 (dd, 1H, H-3), 2.76 (dq, 1H, SC*H*$_2$CH$_3$, J = 7.2 Hz, J = 12.7 Hz), 2.69 (dq,

1H, SCH_2CH_3, J = 7.3 Hz, J = 12.7 Hz), 2.53 (bs, 1H, OH), 1.26 (dd, 3H, $J \sim J$ = 7.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.6, 133.8, 128.9, 128.1, 126.2, 118.0, 103.2, 101.2, 75.2, 70.0, 69.2, 69.0, 68.8, 58.7, 33.1, 14.2; ESI HRMS calc'd for $C_{18}H_{24}O_5S_2Na$: 407.0957. Found: 407.0958.

Preparation of tetanus toxoid and BSA conjugates:

Procedures for synthesis of adipate activated thiooligosaccharides for the preparation of conjugates **124**, **126**, **129** and **131**:

Activation of *S*-GM_3: Amine **52** (10.5 mg; 0.01 mmol) was dissolved in *N,N*-dimethylformamide (1.5 mL) and triethylamine (3.7 μL; 0.02 mmol; 2.2 eq.). The *p*-nitrophenyl diester (0.047 g; 0.12 mmol; 10 eq.) was added, and reaction progress was monitored by TLC (4/5/1/0.5 dichloromethane/methanol/water/acetic acid). After 90 minutes, several drops of acetic acid were added, and the mixture was concentrated at room temperature from toluene (3x) on a rotary evaporator. The residue was suspended in 2% acetic acid solution (3 mL) and filtered through cotton. The filtrate was applied to a SepPak cartridge (0.4 g), washed with 2% acetic acid (10.0 mL), and eluted with 2% acetic acid in methanol. Concentration of the eluent yielded a clear solid which was purified by HPLC (Hamilton PRP-1 semi-preparative column; water-methanol gradient elution) to yield the activated oligosaccharide (0.011 g; 10 μmol; 81 %): ^1H NMR spectra were acquired in CD_3OD. Although visible, resonances corresponding to the Neu5Ac residue were broadened significantly. Selected ^1H NMR resonances are reported here (600 MHz, CD_3OD, δ) 8.29 (m, 2H, Ar), 8.14 (bs, 1H, N*H*), 7.94 (bs, 1H, N*H*), 7.89 (bs, 1H, N*H*), 7.37 (m, 2H, Ar), 5.86 (ddd, 1H, H-4_{sph}, J = 6.7 Hz, J = 10.5 Hz, J = 17.1 Hz), 4.45 (d, 1H, H-1', $J_{1',2'}$ = 7.1 Hz), 4.29 (dd, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 1.99

(bs, 3H, NHC(O)CH_3); ESI HRMS calc'd for $C_{46}H_{70}N_4O_{25}SNa$: 1133.3948. Found: 1133.3948. [Note: addition of an amine (for example diallylamine) to the nmr tube resulted in resolution of the ^{1}H NMR signals corresponding to the Neu5Ac residue].

Activation of *S*-GM_2: Amine **54** (4.5 mg; 4.2 μmol) was dissolved in *N,N*-dimethylformamide (1.5 mL) and triethylamine (2.0 μL; 9.2 μmol; 2.2 eq.). The *p*-nitrophenyl diester (0.017 g; 42 μmol; 10 eq.) was added, and reaction progress was monitored by TLC (4/5/1/0.5 dichloromethane/methanol/water/acetic acid). After 2 hours, several drops of acetic acid were added, and the mixture was concentrated at room temperature from toluene (3x) on a rotary evaporator. The residue was suspended in 2% acetic acid solution (4 mL) and filtered through cotton. The filtrate was applied to a SepPak cartridge (0.4 g), washed with 2% acetic acid (5 mL), and eluted with 2% acetic acid in methanol. Concentration of the eluent yielded a clear solid which was purified by HPLC (Hamilton PRP-1 semi-preparative column; water-methanol gradient elution) to yield the activated thiotetrasaccharide (0.0031 g; 2.3 μmol; 56 %): ^{1}H NMR spectra were acquired in CD_3OD. Although visible, resonances corresponding to the Neu5Ac residue were broadened significantly. Selected ^{1}H NMR resonances are reported here (600 MHz, CD_3OD, δ)) 8.29 (m, 2H, Ar), 8.06 (bd, 1H, N*H*), 7.94 (bt, 1H, N*H*), 7.89 (d, 1H, N*H*), 7.37 (m, 2H, Ar), 5.86 (ddd, 1H, H-4$_{sph}$, J = 6.6 Hz, J = 10.6 Hz, J = 17.3 Hz), 4.63 (bs, 1H, H-1"), 4.43 (d, 1H, H-1', $J_{1',2'}$ = 7.2 Hz), 4.29 (d, 1H, H-1, $J_{1,2}$ = 7.7 Hz), 1.98 (bs, 3H, NHC(O)CH_3); ESI HRMS calc'd for $C_{46}H_{70}N_4O_{25}Sna_1$: 1336.474129. Found: 1336.474246.

Procedure for preparation of TT conjugates 124 and 129 with adipate linker:

Purified tetanus toxoid (~6.6 mg/mL) in PBS, pH 7.2, was added to the activated oligosaccharide (~30 mol. eq.). The mixture was tumbled gently overnight before being dialyzed against PBS (5x2L) at 4 °C.

Procedure for preparation of BSA conjugates 126 and 131 with adipate linker:

Bovine serum albumin (Sigma) (~3.5 mg/mL) in PBS, pH 7.2, was added to the adipate activated oligosaccharide (~30 mol. eq.). The mixture was tumbled gently overnight, dialyzed against milliQ water (5x2L) at 4 °C, and then lyophilized. The glycoconjugate was stored at –20 °C prior to use.

Procedure for preparation of BSA conjugates 127, 128, and 132-136 using diethyl squarate:

Sugar amines (**52-57** and **92**) were dissolved in methanol and 3,4-diethoxy-3-cyclobutene-1,2-dione (0.9 eq.) was added in methanol. Reaction progress was monitored by TLC, and when necessary, sodium methoxide was added. When all starting material had been consumed as judged by TLC, the mixture was transferred to an Ependorph tube and solvent was removed with a stream of argon. The residue was dried *in vacuo* for one hour, after which time a solution of BSA (5 mg/mL) in borate buffer, pH 9.0 was added. The solutions were tumbled overnight and the conjugates processed and stored as described for **126** and **131**.

References

[1] L. A. Lasky, *Ann. Rev. Biochem.* **1995**, *64*, 113-139.

[2] N. E. Zachara, G. W. Hart, *Chem. Rev.* **2002**, *102*, 431-438.

[3] P. M. Rudd, T. Elliott, P. Cresswell, I. A. Wilson, R. A. Dwek, *Science* **2001**, *291*, 2370-2376.

[4] D. J. Miller, R. L. Ax, *Mol. Reprod. Dev.* **1990**, *26*, 184-198.

[5] A. B. Diekman, *Cell. Mol. Life Sci.* **2003**, *60*, 298-308.

[6] E. Ioffe, P. Stanley, *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 728-732.

[7] H. Schachter, *Curr. Opin. Struct. Biol.* **2004**, *14*, 607-616.

[8] R. S. Haltiwanger, J. B. Lowe, *Ann. Rev. Biochem.* **2004**, *73*, 491-537.

[9] J. Masuoka, *Clin. Microbiol. Rev.* **2004**, *17*, 281.

[10] T. W. Rademacher, R. B. Parekh, R. A. Dwek, *Ann. Rev. Biochem.* **1988**, *57*, 785-838.

[11] H. J. Gabius, H. C. Siebert, S. Andre, J. Jimenez-Barbero, H. Rudiger, *Chembiochem* **2004**, *5*, 740-764.

[12] M. Ambrosi, N. R. Cameron, B. G. Davis, *Org. Biomol. Chem.* **2005**, *3*, 1593-1608.

[13] S. Hakomori, *Arch. Biochem. Biophys.* **2004**, *426*, 173-181.

[14] M. Mammen, S. K. Choi, G. M. Whitesides, *Angew. Chem. Int. Edit.* **1998**, *37*, 2755-2794.

[15] K. M. Koeller, C. H. Wong, *Nat. Biotechnol.* **2000**, *18*, 835-841.

[16] O. Hindsgaul, M. Fukuda, *Molecular and Cellular Glycobiology, Vol. 30*, Oxford University Press, **2000**.

[17] D. B. Werz, P. H. Seeberger, *Chem. Eur. J.* **2005**.

[18] H. M. Osborn, P. G. Evans, N. Gemmell, S. D. Osborne, *J. Pharm. Pharmacol.* **2004**, *56*, 691-702.

[19] C. A. Janeway, P. Travers, M. Walport, M. J. Schlomchik, *Immunobiology*, 6th ed., Garland Science Publishing, **2005**.

[20] B. A. Cobb, D. L. Kasper, *Eur. J. Immunol.* **2005**, *35*, 352-356.

[21] T. I. Prigozy, O. Nadenko, P. Qasba, D. Elewaut, L. Brossay, A. Khurana, T. Natori, Y. Koezuka, A. Kulkarni, M. Kronenberg, *Science* **2001**, *291*, 664-667.

[22] L. J. Kobrynski, A. O. Sousa, A. J. Nahmias, F. K. Lee, *J. Immunol.* **2005**, *174*, 1787-1790.

[23] R. C. Kennedy, M. H. Shearer, *Int. Rev. Immunol.* **2003**, *22*, 141-172.

[24] S. Segal, A. J. Pollard, *Br. Med. Bull.* **2005**, *72*, 65-81.

[25] K. L. O'Brien, M. Santosham, *Am. J. Epidemiol.* **2004**, *159*, 634-644.

[26] V. Verez-Bencomo, V. Fernandez-Santana, E. Hardy, M. E. Toledo, M. C. Rodriguez, L. Heynngnezz, A. Rodriguez, A. Baly, L. Herrera, M. Izquierdo, A. Villar, Y. Valdes, K. Cosme, M. L. Deler, M. Montane, E. Garcia, A. Ramos, A. Aguilar, E. Medina, G. Torano, I. Sosa, I. Hernandez, R. Martinez, A. Muzachio, A. Carmenates, L. Costa, F. Cardoso, C. Campa, M. Diaz, R. Roy, *Science* **2004**, *305*, 522-525.

[27] S. Hakomori, *Ann. Rev. Immunol.* **1984**, *2*, 103-126.

[28] T. Feizi, *Nature* **1985**, *314*, 53-57.

[29] W. B. Hamilton, F. Helling, K. O. Lloyd, P. O. Livingston, *Int. J. Cancer* **1993**, *53*, 566-573.

[30] L. Svennerholm, *J. Neurochem.* **1963**, *10*, 613-&.

[31] S. Saha, K. C. Mohanty, *J. Exp. Clin. Cancer Res.* **2003**, *22*, 125-134.

[32] S.-i. Hakomori, Y. Zhang, *Chem. Biol.* **1997**, *4*, 97-104.

[33] P. O. Livingston, M. J. Calves, E. J. Natoli, Jr., *J. Immunol.* **1987**, *138*, 1524-1529.

[34] P. O. Livingston, E. J. Natoli, M. J. Calves, E. Stockert, H. F. Oettgen, L. J. Old, *Proc. Natl. Acad. Sci. U. S. A.* **1987**, *84*, 2911-2915.

[35] F. Helling, A. Shang, M. Calves, S. Zhang, S. Ren, R. K. Yu, H. F. Oettgen, P. O. Livingston, *Cancer Res.* **1994**, *54*, 197-203.

[36] P. B. Chapman, D. Morrisey, K. S. Panageas, L. Williams, J. J. Lewis, R. J. Israel, W. B. Hamilton, P. O. Livingston, *Clin. Cancer Res.* **2000**, *6*, 4658-4662.

[37] H. Zhang, S. Zhang, N. K. Cheung, G. Ragupathi, P. O. Livingston, *Cancer Res.* **1998**, *58*, 2844-2849.

[38] P. O. Livingston, G. Y. Wong, S. Adluri, Y. Tao, M. Padavan, R. Parente, C. Hanlon, M. J. Calves, F. Helling, G. Ritter, et al., *J. Clin. Oncol.* **1994**, *12*, 1036-1044.

[39] P. O. Livingston, G. Ragupathi, C. Musselli, *J. Clin. Immunol.* **2000**, *20*, 85-93.

[40] C. Musselli, P. O. Livingston, G. Ragupathi, *J. Cancer Res. Clin. Oncol.* **2001**, *127 Suppl 2*, R20-26.

[41] T. Carmenate, L. Canaan, A. Alvarez, M. Delgado, S. Gonzalez, T. Menendez, L. Rodes, G. Guillen, *FEMS Immunol. Med. Microbiol.* **2004**, *40*, 193-199.

[42] A. Fattom, X. Li, Y. H. Cho, A. Burns, A. Hawwari, S. E. Shepherd, R. Coughlin, S. Winston, R. Naso, *Vaccine* **1995**, *13*, 1288-1293.

[43] G. Ragupathi, M. Meyers, S. Adluri, L. Howard, C. Musselli, P. O. Livingston, *Int. J. Cancer* **2000**, *85*, 659-666.

[44] Y. Pan, P. Chefalo, N. Nagy, C. Harding, Z. Guo, *J. Med. Chem.* **2005**, *48*, 875-883.

[45] R. A. Pon, M. Lussier, Q. L. Yang, H. J. Jennings, *J. Exp. Med.* **1997**, *185*, 1929-1938.

[46] W. Zou, S. Borrelli, M. Gilbert, T. Liu, R. A. Pon, H. J. Jennings, *J. Biol. Chem.* **2004**, *279*, 25390-25399.

[47] E. Bolesta, A. Kowalczyk, A. Wierzbicki, P. Rotkiewicz, B. Bambach, C. Y. Tsao, I. Horwacik, A. Kolinski, H. Rokita, M. Brecher, X. Wang, S. Ferrone, D. Kozbor, *Cancer Res.* **2005**, *65*, 3410-3418.

[48] G. Cunto-Amesty, P. Luo, B. Monzavi-Karbassi, A. Lees, T. Kieber-Emmons, *Vaccine* **2001**, *19*, 2361.

[49] F. Hudecz, *Biologicals* **2001**, *29*, 197-207.

[50] J. P. Meyer, T. J. Gillespie, S. Hom, V. J. Hruby, T. P. Davis, *Peptides* **1995**, *16*, 1215-1219.

[51] R. Tugyi, K. Uray, D. Ivan, E. Fellinger, A. Perkins, F. Hudecz, *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 413-418.

[52] D. Steer, R. Lew, P. Perlmutter, A. I. Smith, M. I. Aguilar, *Biochemistry* **2002**, *41*, 10819-10826.

[53] D. Urban, T. Skrydstrup, J. M. Beau, *Chem. Commun.* **1998**, 955-956.

[54] E. Bousquet, A. Spadaro, M. S. Pappalardo, R. Bernardini, R. Romeo, L. Panza, G. Ronsisvalle, *J. Carbohydr. Chem.* **2000**, *19*, 527-541.

[55] L. Cipolla, M. Rescigno, A. Leone, F. Peri, B. La Ferla, F. Nicotra, *Bioorg. Med. Chem.* **2002**, *10*, 1639-1646.

[56] B. Kuberan, S. A. Sikkander, H. Tomiyama, R. J. Linhardt, *Angew. Chem. Int. Edit.* **2003**, *42*, 2073-2075.

[57] S. V. Evans, C. R. MacKenzie, *J. Mol. Recognit.* **1999**, *12*, 155-168.

[58] N. Papini, L. Anastasia, C. Tringali, G. Croci, R. Bresciani, K. Yamaguchi, T. Miyagi, A. Preti, A. Prinetti, S. Prioni, S. Sonnino, G. Tettamanti, B. Venerando, E. Monti, *J. Biol. Chem.* **2004**, *279*, 16989-16995.

[59] J. Kopitz, C. Oehler, M. Cantz, *FEBS Lett.* **2001**, *491*, 233-236.

[60] T. Miyagi, T. Wada, A. Iwamatsu, K. Hata, Y. Yoshikawa, S. Tokuyama, M. Sawada, *J. Biol. Chem.* **1999**, *274*, 5004-5011.

[61] E. Montero, A. Garcia-Herrero, J. L. Asensio, K. Hirai, S. Ogawa, F. Santoyo-Gonzalez, F. J. Canada, J. Jimenez-Barbero, *Eur. J. Org. Chem.* **2000**, 1945-1952.

[62] H. Yuasa, H. Hashimoto, *Trends Glycosci. Glycotech.* **2001**, *13*, 31-55.

[63] B. J. Smith, *J. Mol. Graph.* **2003**, *22*, 151-159.

[64] D. Horton, D. H. Hutson, *Adv. Carbohydr. Chem.* **1963**, *18*, 123-199.

[65] I. Goodman, J. R. Fouts, E. Bresnick, R. Menegas, G. H. Hitchings, *Science* **1959**, *130*, 450-451.

[66] C. S. Rye, S. G. Withers, *Carbohydr. Res.* **2004**, *339*, 699-703.

[67] W. P. Burmeister, S. Cottaz, H. Driguez, R. Iori, S. Palmieri, B. Henrissat, *Structure* **1997**, *5*, 663-675.

[68] S. Cottaz, B. Henrissat, H. Driguez, *Biochemistry* **1996**, *35*, 15256-15259.

[69] A. Bourderioux, M. Lefoix, D. Gueyrard, A. Tatibouet, S. Cottaz, S. Arzt, W. P. Burmeister, P. Rollin, *Org. Biomol. Chem.* **2005**, *3*, 1872-1879.

[70] M. Blancmuesser, J. Defaye, H. Driguez, *Carbohydr. Res.* **1978**, *67*, 305-328.

[71] J. K. Fairweather, H. Driguez, in *Carbohydrates in Chemistry and Biology, Vol. 1* (Eds.: B. Ernst, G. W. Hart, P. Sinay), Wiley-VCH, **2000**, pp. 531-564.

[72] J. Defaye, J. Gelas, in *Studies in Natural Products Chemistry, Vol. 8* (Ed.: Atta-ur-Rahman), Elsevier, Oxford, **1991**, pp. 315-357.

[73] Z. J. Witczak, J. M. Sun, R. Mielguj, *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2169-2174.

[74] H. Hashimoto, K. Shimada, S. Horito, *Tetrahedron: Asymmetry* **1994**, *5*, 2351-2366.

[75] M. Jahn, J. Marles, R. A. J. Warren, S. G. Withers, *Angew. Chem. Int. Edit.* **2003**, *42*, 352-+.

[76] M. Jahn, H. M. Chen, J. Mullegger, J. Marles, R. A. J. Warren, S. G. Withers, *Chem. Commun.* **2004**, 274-275.

[77] H. Driguez, in *Glycoscience: Synthesis of Substrate Analogues and Mimetics, Vol. 187* (Eds.: J. Thiem, H. Driguez), Springer, **1997**, pp. 85-116.

[78] L. X. Wang, N. Sakairi, H. Kuzuhara, *J. Carbohydr. Chem.* **1990**, *9*, 441-450.

[79] J. Defaye, H. Driguez, E. Ohleyer, C. Orgeret, C. Viet, *Carbohydr. Res.* **1984**, *130*, 317-321.

[80] A. Gadelle, J. Defaye, C. Pedersen, *Carbohydr. Res.* **1990**, *200*, 497-498.

[81] S. B. Cohen, R. L. Halcomb, *J. Org. Chem.* **2000**, *65*, 6145-6152.

[82] W. Xu, S. A. Springfield, J. T. Koh, *Carbohydr. Res.* **2000**, *325*, 169-176.

[83] S. J. Williams, S. G. Withers, *Aust. J. Chem.* **2002**, *55*, 3-12.

[84] D. H. G. Crout, G. Vic, *Curr. Opin. Chem. Biol.* **1998**, *2*, 98-111.

[85] G. Vic, D. H. G. Crout, *Tetrahedron: Asymmetry* **1994**, *5*, 2513-2516.

[86] G. H. Meulenbeld, B. M. De Roode, S. Hartmans, *Biocatal. Biotransform.* **2002**, *20*, 251-256.

[87] D. E. Koshland, *Biol. Rev. Cambridge Philosophic. Soc.* **1953**, *28*, 416-436.

[88] R. V. Stick, K. A. Stubbs, *Tetrahedron: Asymmetry* **2005**, *16*, 321-335.

[89] H. Driguez, *Chembiochem* **2001**, *2*, 311-318.

[90] Z. J. Witczak, *Curr. Med. Chem.* **1999**, *6*, 165-178.

[91] G. Sulzenbacher, H. Driguez, B. Henrissat, M. Schulein, G. J. Davies, *Biochemistry* **1996**, *35*, 15280-15287.

[92] E. Montero, M. Vallmitjana, J. A. Perez-Pons, E. Querol, J. Jimenez-Barbero, F. J. Canada, *FEBS Lett.* **1998**, *421*, 243-248.

[93] M. Qian, S. Spinelli, H. Driguez, F. Payan, *Protein Sci.* **1997**, *6*, 2285-2296.

[94] C. Reverbel-Leroy, G. Parsiegla, V. Moreau, M. Juy, C. Tardif, H. Driguez, J. P. Belaich, R. Haser, *Acta Crystallogr. D Biol. Crystallogr.* **1998**, *54 (Pt 1)*, 114-118.

[95] D. Mandelman, A. Belaich, J. P. Belaich, N. Aghajari, H. Driguez, R. Haser, *J. Bacteriol.* **2003**, *185*, 4127-4135.

[96] G. Parsiegla, C. Reverbel-Leroy, C. Tardif, J. P. Belaich, H. Driguez, R. Haser, *Biochemistry* **2000**, *39*, 11238-11246.

[97] G. Parsiegla, M. Juy, C. Reverbel-Leroy, C. Tardif, J. P. Belaich, H. Driguez, R. Haser, *EMBO J* **1998**, *17*, 5551-5562.

[98] Z. J. Witczak, D. Boryczewski, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3265-3268.

[99] M. Blanc-Muesser, L. Vigne, H. Driguez, J. Lehmann, J. Steck, K. Urbahns, *Carbohydr. Res.* **1992**, *224*, 59-71.

[100] G. Legler, *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 319-384.

[101] C. Schou, G. Rasmussen, M. Schulein, B. Henrissat, H. Driguez, *J. Carbohydr. Chem.* **1993**, *12*, 743-752.

[102] Y. Suzuki, K. Sato, M. Kiso, A. Hasegawa, *Glycoconj. J.* **1990**, *7*, 349-354.

[103] C. Orgeret, E. Seillier, C. Gautier, J. Defaye, H. Driguez, *Carbohydr. Res.* **1992**, *224*, 29-40.

[104] D. Rho, M. Desrochers, L. Jurasek, H. Driguez, J. Defaye, *J. Bacteriol.* **1982**, *149*, 47-53.

[105] R. Schauer, S. Kelm, G. Reuter, P. Roggentin, L. Shaw, in *Biology of the Sialic Acids* (Ed.: A. Rosenberg), Plenum Press, New York, **1995**.

[106] P. J. Garegg, *Adv. Carbohydr. Chem. Biochem.* **2004**, *59*, 69-134.

[107] H. Paulsen, *Angew. Chem. Int. Edit. Engl.* **1982**, *21*, 155-173.

[108] Y. T. Li, S. C. Li, H. Ishida, M. Kiso, L. Raimondi, A. Bernardi, S. Sonnino, *Methods Enzymol.* **2003**, *363*, 242-264.

[109] S. Sonnino, P. Brocca, D. Acquotti, A. Bernardi, L. Raimondi, M. Kiso, H. Ishida, S. C. Li, Y. T. Li, *Biosci. Rep.* **1999**, *19*, 163-168.

[110] Y. T. Li, S. C. Li, A. Hasegawa, H. Ishida, M. Kiso, A. Bernardi, P. Brocca, L. Raimondi, S. Sonnino, *J. Biol. Chem.* **1999**, *274*, 10014-10018.

[111] T. Taki, K. Ishii, A. Myoga, in *Gangliosides and Cancer* (Ed.: H. F. Oettgen), VCH, New York, **1989**, pp. 129-135.

[112] R. Dawes, K. M. Gough, P. G. Hultin, *J. Phys. Chem. A* **2005**, *109*, 213-217.

[113] R. Dawes, K. M. Gough, P. G. Hultin, *J. Phys. Chem. A* **2005**, *109*, 218-223.

[114] A. Hasegawa, J. Nakamura, M. Kiso, *J. Carbohydr. Chem.* **1986**, *5*, 21-31.

[115] A. Hasegawa, M. Morita, Y. Ito, H. Ishida, M. Kiso, *J. Carbohydr. Chem.* **1990**, *9*, 369-392.

[116] A. Hasegawa, J. Nakamura, M. Kiso, *J. Carbohydr. Chem.* **1986**, *5*, 11-19.

[117] S. Bennett, M. Von Itzstein, M. J. Kiefel, *Carbohydr. Res.* **1994**, *259*, 293-299.

[118] M. J. Kiefel, B. Beisner, S. Bennett, I. D. Holmes, M. von Itzstein, *J. Med. Chem.* **1996**, *39*, 1314-1320.

[119] G. Hummel, O. Hindsgaul, *Angew. Chem. Int. Edit.* **1999**, *38*, 1782-1784.

[120] D. X. Qiu, R. R. Schmidt, *Liebigs Ann.* **1992**, 217-224.

[121] S. Hanessian, N. R. Plessas, *J. Org. Chem.* **1969**, *34*, 1035-&.

[122] K. Jansson, S. Ahlfors, T. Frejd, J. Kihlberg, G. Magnusson, J. Dahmen, G. Noori, K. Stenvall, *J. Org. Chem.* **1988**, *53*, 5629-5647.

[123] P. J. Garegg, B. Samuelsson, *J. Chem. Soc. Perkin Trans. 1* **1980**, 2866-2869.

[124] P. J. Garegg, B. Samuelsson, *J. Chem. Soc. Chem. Commun.* **1979**, 978-980.

[125] O. Kanie, J. Nakamura, Y. Itoh, M. Kiso, A. Hasegawa, *J. Carbohydr. Chem.* **1987**, *6*, 117-128.

[126] T. Eisele, R. R. Schmidt, *Liebigs Ann. Recl.* **1997**, 865-872.

[127] J. C. Wilson, M. J. Kiefel, D. I. Angus, M. von Itzstein, *Org. Lett.* **1999**, *1*, 443-446.

[128] W. B. Turnbull, R. A. Field, *J. Chem. Soc. Perkin Trans. 1* **2000**, 1859-1866.

[129] W. B. Turnbull, J. A. Harrison, K. P. R. Kartha, S. Schenkman, R. A. Field, *Tetrahedron* **2002**, *58*, 3207-3216.

[130] R. Kuhn, P. Lutz, Macdonald. L, *Chem. Ber.-Recl.* **1966**, *99*, 611-&.

[131] L. A. Mulard, P. Kovac, C. P. J. Glaudemans, *Carbohydr. Res.* **1994**, *259*, 21-34.

[132] K. Kohata, S. A. Abbas, K. L. Matta, *Carbohydr. Res.* **1984**, *132*, 127-135.

[133] H. M. Flowers, *Carbohydr. Res.* **1975**, *39*, 245-251.

[134] M. Nitz, PhD thesis, University of Alberta **2001**.

[135] R. Lattrell, G. Lohaus, *Annalen Der Chemie-Justus Liebig* **1974**, 901-920.

[136] R. Albert, K. Dax, R. W. Link, A. E. Stutz, *Carbohydr. Res.* **1983**, *118*, C5-C6.

[137] R. W. Binkley, *J. Carbohydr. Chem.* **1994**, *13*, 111-123.

[138] S. David, S. Hanessian, *Tetrahedron* **1985**, *41*, 643-663.

[139] A. Hasegawa, T. Ando, A. Kameyama, M. Kiso, *J. Carbohydr. Chem.* **1992**, *11*, 645-658.

[140] T. L. Lowary, E. Eichler, D. R. Bundle, *J. Org. Chem.* **1995**, *60*, 7316-7327.

[141] W. Trahanov, L. B. Young, *J. Chem.Soc.* **1965**, 5777-&.

[142] R. Johansson, B. Samuelsson, *J. Chem. Soc. Perkin Trans. 1* **1984**, 2371-2374.

[143] A. M. Horneman, I. Lundt, *Synthesis* **1999**, 317-325.

[144] C. Copeland, R. V. Stick, *Aust. J. Chem.* **1978**, *31*, 1371-1374.

[145] R. R. Schmidt, *Angew. Chem. Int. Edit. Engl.* **1986**, *25*, 212-235.

[146] R. R. Schmidt, W. Klotz, *Synlett* **1991**, 168-170.

[147] W. K. C. Park, S. J. Meunier, R. Roy, *Carbohydr. Lett.* **1995**, *1*, 179-184.

[148] D. Beith-Halahmi, H. M. Flowers, D. Shapiro, *Carbohydr. Res.* **1967**, *5*, 25-30.

[149] I. A. Ivanova, A. V. Nikolaev, *J. Chem. Soc. Perkin Trans. 1* **1998**, 3093-3099.

[150] M. Blancmuesser, J. Defaye, H. Driguez, *J. Chem. Soc. Perkin Trans. 1* **1982**, 15-18.

[151] T. Endo, K. Oda, Mukaiyam.T, *Chem. Lett.* **1974**, 443-444.

[152] O. B. Wallace, D. M. Springer, *Tetrahedron Lett.* **1998**, *39*, 2693-2694.

[153] K. E. Yelm, *Tetrahedron Lett.* **1999**, *40*, 1101-1102.

[154] J. F. Batey, C. Bullock, E. Obrien, J. M. Williams, *Carbohydr. Res.* **1975**, *43*, 43-50.

[155] P. J. Garegg, H. Hultberg, *Carbohydr. Res.* **1982**, *110*, 261-266.

[156] K. Hotta, H. Ishida, M. Kiso, A. Hasegawa, *J. Carbohydr. Chem.* **1994**, *13*, 175-191.

[157] A. Liakatos, M. J. Kiefel, M. von Itzstein, *Org. Lett.* **2003**, *5*, 4365-4368.

[158] G. J. Boons, A. V. Demchenko, *Chem. Rev.* **2000**, *100*, 4539-4566.

[159] H. Ando, Y. Koike, H. Ishida, M. Kiso, *Tetrahedron Lett.* **2003**, *44*, 6883-6886.

[160] Y. Kajihara, H. Kodama, T. Endo, H. Hashimoto, *Carbohydr. Res.* **1998**, *306*, 361-378.

[161] M. Izumi, G. J. Shen, S. Wacowich-Sgarbi, T. Nakatani, O. Plettenburg, C. H. Wong, *J. Am. Chem. Soc.* **2001**, *123*, 10909-10918.

[162] W. Zou, M. Abraham, M. Gilbert, W. W. Wakarchuk, H. J. Jennings, *Glycoconj. J.* **1999**, *16*, 507-515.

[163] G. T. Hermanson, *Bioconjugate Techniques*, Academic Press, New York, **1996**.

[164] S. Brodesser, P. Sawatzki, T. Kolter, *Eur. J. Org. Chem.* **2003**, 2021-2034.

[165] S. V. Vasudevan, P. V. Balaji, *J. Phys. Chem. B* **2001**, *105*, 7033-7041.

[166] D. R. Bundle, C. C. Ling, P. Zhang, PCT Designated States, **2003**.

[167] L. Liu, PhD thesis, University of Alberta **2003**.

[168] H. Hasegawa, T. Yamamoto, S. Hatano, T. Hakogi, S. Katsumura, *Chem. Lett.* **2004**, *33*, 1592-1593.

[169] A. N. Rai, A. Basu, *Org. Lett.* **2004**, *6*, 2861-2863.

[170] S. Torssell, P. Somfai, *Org. Biomol. Chem.* **2004**, *2*, 1643-1646.

[171] P. Zimmermann, R. Bommer, T. Bar, R. R. Schmidt, *J. Carbohydr. Chem.* **1988**, *7*, 435-452.

[172] Y. D. Vankar, R. R. Schmidt, *Chem. Soc. Rev.* **2000**, *29*, 201-216.

[173] S. J. Danishefsky, J. R. Allen, *Angew. Chem. Int. Edit.* **2000**, *39*, 836-863.

[174] H. Ishida, M. Kiso, *Trends Glycosci. Glycotechnol.* **2001**, *13*, 57-64.

[175] S. Fujita, M. Numata, M. Sugimoto, K. Tomita, T. Ogawa, *Carbohydr. Res.* **1994**, *263*, 181-196.

[176] D. K. Ress, R. J. Linhardt, *Curr. Org. Synth.* **2004**, *1*, 31-46.

[177] M. A. Peterson, R. Polt, *J. Org. Chem.* **1993**, *58*, 4309-4314.

[178] T. Murase, H. Ishida, M. Kiso, A. Hasegawa, *Carbohydr. Res.* **1988**, *184*, c1-c4.

[179] M. M. Palcic, *Curr. Opin. Biotechnol.* **1999**, *10*, 616-624.

[180] S. Hanson, M. Best, M. C. Bryan, C. H. Wong, *Trends Biochem.Sci.* **2004**, *29*, 656-663.

[181] P. L. DeAngelis, *Glycobiology* **2002**, *12*, 9R-16.

[182] S. Koizumi, *Trends Glycosci. Glycotechnol.* **2003**, *15*, 65-74.

[183] M. Gilbert, J. R. Brisson, M. F. Karwaski, J. Michniewicz, A. M. Cunningham, Y. Y. Wu, N. M. Young, W. W. Wakarchuk, *J. Biol. Chem.* **2000**, *275*, 3896-3906.

[184] K. F. Johnson, *Glycoconj. J.* **1999**, *16*, 141-146.

[185] R. M. Mizanur, C. J. Zea, N. L. Pohl, *J. Am. Chem. Soc.* **2004**, *126*, 15993-15998.

[186] J. C. Errey, B. Mukhopadhyay, K. P. R. Kartha, R. A. Field, *Chem. Commun.* **2004**, 2706-2707.

[187] J. Yang, X. Fu, Q. Jia, J. Shen, J. B. Biggins, J. Q. Jiang, J. J. Zhao, J. J. Schmidt, P. G. Wang, J. S. Thorson, *Org. Lett.* **2003**, *5*, 2223-2226.

[188] J. Q. Jiang, J. B. Biggins, J. S. Thorson, *J. Am. Chem. Soc.* **2000**, *122*, 6803-6804.

[189] M. Gilbert, D. C. Watson, A. M. Cunningham, M. P. Jennings, N. M. Young, W. W. Wakarchuk, *J. Biol. Chem.* **1996**, *271*, 28271-28276.

[190] B. Priem, M. Gilbert, W. W. Wakarchuk, A. Heyraud, E. Samain, *Glycobiology* **2002**, *12*, 235-240.

[191] T. Antoine, A. Heyraud, C. Bosso, E. Samain, *Angew. Chem. Int. Edit.* **2005**, *44*, 1350-1352.

[192] T. Antoine, B. Priem, A. Heyraud, L. Greffe, M. Gilbert, W. W. Wakarchuk, J. S. Lam, E. Samain, *Chembiochem* **2003**, *4*, 406-412.

[193] S. Fort, L. Birikaki, M.-P. Dubois, T. Antoine, E. Samain, H. Driguez, *Chem. Commun.* **2005**, 2558-2560.

[194] Griesbau.K, *Angew. Chem. Int. Edit.* **1970**, *9*, 273-&.

[195] P. B. van Seeventer, J. A. L. M. van Dorst, J. F. Siemerink, J. P. Kamerling, J. F. G. Vliegenthart, *Carbohydr. Res.* **1997**, *300*, 369-373.

[196] Y. Lapidot, S. Rappaport, Y. Wolman, *J. Lipid Res.* **1967**, *8*, 142-145.

[197] A. K. Chatterjee, R. H. Grubbs, *Org. Lett.* **1999**, *1*, 1751-1753.

[198] S. B. Garber, J. S. Kingsbury, B. L. Gray, A. H. Hoveyda, *J. Am. Chem. Soc.* **2000**, *122*, 8168-8179.

[199] D. P. Larson, C. H. Heathcock, *J. Org. Chem.* **1996**, *61*, 5208-5209.

[200] P. J. Garegg, H. Hultberg, *Carbohydr. Res.* **1981**, *93*, C10-C11.

[201] O. W. Lever, B. R. Vestal, *J. Heterocycl. Chem.* **1986**, *23*, 901-903.

[202] E. J. Corey, J. W. Suggs, *J. Org. Chem.* **1973**, *38*, 3224-3224.

[203] A. B. Smith, R. A. Rivero, K. J. Hale, H. A. Vaccaro, *J. Am. Chem. Soc.* **1991**, *113*, 2092-2112.

[204] R. Ohrlein, *Biocatalysis - from Discovery to Application* **1999**, *200*, 227-254.

[205] M. M. Palcic, O. Hindsgaul, *Trends Glycosci. Glycotechnol.* **1996**, *8*, 37-49.

[206] Y. L. Ding, O. Hindsgaul, H. Li, M. M. Palcic, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3199-3202.

[207] P. P. Lu, O. Hindsgaul, H. Li, M. M. Palcic, *Can. J. Chem.-Rev. Can. Chim.* **1997**, *75*, 790-800.

[208] V. Moreau, J. L. Viladot, E. Samain, A. Planas, H. Driguez, *Bioorg. Med. Chem.* **1996**, *4*, 1849-1855.

[209] V. Moreau, H. Driguez, *J. Chem. Soc.-Perkin Trans. 1* **1996**, 525-527.

[210] E. Samain, C. Lancelonpin, F. Ferigo, V. Moreau, H. Chanzy, A. Heyraud, H. Driguez, *Carbohydr. Res.* **1995**, *271*, 217-226.

[211] A. M. Southwick, L. X. Wang, S. R. Long, Y. C. Lee, *J. Bacteriol.* **2002**, *184*, 4039-4043.

[212] S. Bernatchez, C. M. Szymanski, N. Ishiyama, J. Li, H. C. Jarrell, P. C. Lau, A. M. Berghuis, N. M. Young, W. W. Wakarchuk, *J. Biol. Chem.* **2005**, *280*, 4792-4802.

[213] O. Blixt, D. Vasiliu, K. Allin, N. Jacobsen, D. Warnock, N. Razi, J. C. Paulson, S. Bernatchez, M. Gilbert, W. W. Wakarchuk, *Carbohydr. Res.* **2005**, *340*, 1963-1972.

[214] J. Ohlsson, G. Magnusson, *Tetrahedron* **2000**, *56*, 9975-9984.

[215] M.-F. Karwaski, W. W. Wakarchuk, M. Gilbert, *Protein Express. Purif.* **2002**, *25*, 237-240.

[216] S. Jacques, J. R. Rich, D. R. Bundle, *Org. Biomol. Chem.* **2005**, *manuscript in preparation.*

[217] S. Mehta, M. Gilbert, W. W. Wakarchuk, D. M. Whitfield, *Org. Lett.* **2000**, *2*, 751-753.

[218] M. Sakagami, K. Horie, K. Higashi, H. Yamada, H. Hamana, *Chem. Pharm. Bull.* **1999**, *47*, 1237-1245.

[219] A. Hasegawa, H. Ogawa, M. Kiso, *J. Carbohydr. Chem.* **1991**, *10*, 1009-1021.

[220] T. Bar, R. R. Schmidt, *Liebigs Ann.* **1991**, 185-187.

[221] H. A. Nunez, R. Barker, *Biochemistry* **1980**, *19*, 489-495.

[222] C. H. Wong, S. L. Haynie, G. M. Whitesides, *J. Org. Chem.* **1982**, *47*, 5416-5418.

[223] C. Auge, S. David, C. Mathieu, C. Gautheron, *Tetrahedron Lett.* **1984**, *25*, 1467-1470.

[224] S. Sabesan, J. Paulson, *J. Am. Chem. Soc.* **1986**, *108*, 2068-2080.

[225] V. P. Kamath, R. E. Yeske, J. M. Gregson, R. M. Ratcliffe, Y. R. Fang, M. M. Palcic, *Carbohydr. Res.* **2004**, *339*, 1141-1146.

[226] Y. R. Fang, K. Sujino, A. Lu, J. Gregson, R. Yeske, V. P. Kamath, R. M. Ratcliffe, M. J. Schur, W. W. Wakarchuk, M. M. Palcic, in *Carbohydrate Bioengineering, Interdisciplinary Approaches* (Eds.: T. T. Teeri, B. Svensson, H. J. Gilbert, T. Feizi), Royal Society of Chemistry, London, **2002**, pp. 127-134.

[227] P. M. Coutinho, E. Deleury, G. J. Davies, B. Henrissat, *J. Mol. Biol.* **2003**, *328*, 307-317.

[228] X. P. Qian, K. Sujino, M. M. Palcic, R. M. Ratcliffe, *J. Carbohydr. Chem.* **2002**, *21*, 911-942.

[229] J. Elhalabi, K. G. Rice, *Carbohydr. Res.* **2001**, *335*, 159-165.

[230] J. Elhalabi, K. G. Rice, *Carbohydr. Res.* **2002**, *337*, 1935-1940.

[231] J. Elhalabi, K. G. Rice, *Nucleos. Nucleot. Nucleic Acids* **2004**, *23*, 195-205.

[232] L. Bornaghi, J. P. Utille, E. D. Rekai, J. M. Mallet, P. Sinay, H. Driguez, *Carbohydr. Res.* **1997**, *305*, 561-568.

[233] O. Tsuruta, H. Yuasa, H. Hashimoto, K. Sujino, A. Otter, H. Li, M. M. Palcic, *J. Org. Chem.* **2003**, *68*, 6400-6406.

[234] Y. Kajihara, H. Kodama, T. Wakabayashi, K. Sato, H. Hashimoto, *Carbohydr. Res.* **1993**, *247*, 179-193.

[235] R. U. Lemieux, in *VIIIth International Symposium on Medicinal Chemistry, Proceedings, Vol. 1*, Swedish Pharmaceutical Press, Stockholm, Uppsala, Sweden, **1984**, pp. 329-351.

[236] O. Hindsgaul, K. J. Kaur, G. Srivastava, M. Blaszczyk-Thurin, S. C. Crawley, L. D. Heerze, M. M. Palcic, *J. Biol. Chem.* **1991**, *266*, 17858-17862.

[237] R. U. Lemieux, *Chem. Soc. Rev.* **1989**, *18*, 347-374.

[238] K. Sujino, C. Malet, O. Hindsgaul, M. M. Palcic, *Carbohydr. Res.* **1998**, *305*, 483-489.

[239] W. Blanken, D. Van den Eijnden, *J. Biol. Chem.* **1985**, *260*, 12927-12934.

[240] M. M. Palcic, K. Sujino, *Trends Glycosci. Glycotechnol.* **2001**, *13*, 361-370.

[241] M. M. Palcic, L. D. Heerze, M. Pierce, O. Hindsgaul, *Glycoconj. J.* **1988**, *5*, 49-63.

[242] M. Karplus, *J. Am. Chem. Soc.* **1963**, *85*, 2870-2871.

[243] J. K. Baker, *Spectrosc. Lett.* **1992**, *25*, 31-48.

[244] I. Tvaroska, K. Mazeau, M. Blanc-Muesser, S. Lavaitte, H. Driguez, F. R. Taravel, *Carbohydr. Res.* **1992**, *229*, 225-231.

[245] J. Fang, X. Chen, W. Zhang, A. Janczuk, P. G. Wang, *Carbohydr. Res.* **2000**, *329*, 873-878.

[246] K. Sujino, C. Malet, O. Hindsgaul, M. M. Palcic, *Carbohydr. Res.* **1997**, *305*, 483-489.

[247] A. K. Datta, R. Chammas, J. C. Paulson, *J. Biol. Chem.* **2001**, *276*, 15200-15207.

[248] W. W. Wakarchuk, A. Cunningham, D. C. Watson, N. M. Young, *Protein Eng.* **1998**, *11*, 295-302.

[249] O. Blixt, I. van Die, T. Norberg, D. H. van den Eijnden, *Glycobiology* **1999**, *9*, 1061-1071.

[250] A. Y. Chernyak, K. V. Antonov, N. K. Kochetkov, *Carbohydr. Res.* **1985**, *141*, 199-212.

[251] F. Dasgupta, L. Anderson, *Carbohydr. Res.* **1994**, *264*, 155-160.

[252] M. M. Palcic, N. O. L. Seto, O. Hindsgaul, *Transfusion Med.* **2001**, *11*, 315-323.

[253] T. L. Lowary, O. Hindsgaul, *Carbohydr. Res.* **1993**, *249*, 163-195.

[254] R. U. Lemieux, K. B. Hendriks, R. V. Stick, K. James, *J. Am. Chem. Soc.* **1975**, *97*, 4056-4062.

[255] Y. Nishida, Y. Shingu, H. Dohi, K. Kobayashi, *Org. Lett.* **2003**, *5*, 2377-2380.

[256] A. G. Myers, J. Liang, M. Hammond, *Tetrahedron Lett.* **1999**, *40*, 5129-5133.

[257] J.-P. Briand, N. Benkirane, G. Guichard, J. F. E. Newman, M. H. V. Van Regenmortel, F. Brown, S. Muller, *Proc. Natl. Acad. Sci. U. S. A.* **1997**, *94*, 12545-12550.

[258] G. Guichard, N. Benkirane, G. Zeder-Lutz, M. Regenmortel, J. Briand, S. Muller, *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 9765-9769.

[259] D. R. Bundle, J. R. Rich, S. Jacques, H. N. Yu, M. Nitz, *Angew. Chem. Int. Edit.* **2005**, *accepted*.

[260] V. Kudryashov, P. W. Glunz, L. J. Williams, S. Hintermann, S. J. Danishefsky, K. O. Lloyd, *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 3264-3269.

[261] F. Mawas, J. Niggemann, C. Jones, M. J. Corbel, J. P. Kamerling, J. F. G. Vliegenthart, *Infect. Immun.* **2002**, *70*, 5107-5114.

[262] T. Buskas, Y. Li, G. J. Boons, *Chem. Eur. J.* **2004**, *10*, 3517-3524.

[263] J. M. Peeters, T. G. Hazendonk, E. C. Beuvery, G. I. Tesser, *J. Immunol. Methods* **1989**, *120*, 133-143.

[264] G. Y. Ishioka, A. G. Lamont, D. Thomson, N. Bulbow, F. C. Gaeta, A. Sette, H. M. Grey, *J. Immunol.* **1992**, *148*, 2446-2451.

[265] E. G. Leonard, D. H. Canaday, C. V. Harding, J. R. Schreiber, *Infect Immun* **2003**, *71*, 4186-4189.

[266] R. J. May, D. O. Beenhouwer, M. D. Scharff, *J Immunol* **2003**, *171*, 4905-4912.

[267] D. E. Tsvetkov, P. E. Cheshev, A. B. Tuzikov, A. A. Chinarev, G. V. Pazynina, M. A. Sablina, A. S. Gambaryan, N. V. Bovin, R. Rieben, A. S. Shashkov, N. E. Nifant'ev, *Russ. J. Bioorg. Chem.* **2002**, *28*, 470-486.

[268] X. Y. Wu, C. C. Ling, D. R. Bundle, *Org. Lett.* **2004**, *6*, 4407-4410.

[269] M. Lahmann, L. Bulow, P. Teodorovic, H. Gyback, S. Oscarson, *Glycoconj. J.* **2004**, *21*, 251-256.

[270] V. P. Kamath, P. Diedrich, O. Hindsgaul, *Glycoconj. J.* **1996**, *13*, 315-319.

[271] M. Kotani, H. Ozawa, I. Kawashima, S. Ando, T. Tai, *Biochim. Biophys. Acta* **1992**, *1117*, 97-103.

[272] D. R. Bundle, in *Carbohydrates* (Ed.: S. Hecht), Oxford University Press, Oxford, **1998**, pp. 370-440.

[273] S. M. Andersen, C. C. Ling, P. Zhang, K. Townson, H. J. Willison, D. R. Bundle, *Org. Biomol. Chem.* **2004**, *2*, 1199-1212.

[274] J. R. Rich, D. R. Bundle, *Org. Lett.* **2004**, *6*, 897-900.

[275] J. R. Rich, A. Szpacenko, M. M. Palcic, D. R. Bundle, *Angew. Chem. Int. Edit.* **2004**, *43*, 613-615.

[276] J. R. Rich, W. W. Wakarchuk, D. R. Bundle, *Chem. Eur. J.* **2005**, *accepted*.

[277] A. Geyer, G. Hummel, T. Eisele, S. Reinhardt, R. R. Schmidt, *Chem. Eur. J.* **1996**, *2*, 981-988.

[278] S. Jacobsen, O. Mols, *Acta Chem. Scand. Ser. B* **1981**, *35*, 163-168.

[279] H. H. Baer, S. A. Abbas, *Carbohydr. Res.* **1980**, *84*, 53-60.

[280] J. Ohlsson, G. Magnusson, *Tetrahedron Lett.* **1999**, *40*, 2011-2014.

[281] H. L. Jiao, O. Hindsgaul, *J. Carbohydr. Chem.* **1999**, *18*, 499-513.

[282] U. Spohr, R. U. Lemieux, *Carbohydr. Res.* **1988**, *174*, 211-237.

[283] T. Mukaiyama, K. Takahashi, *Tetrahedron Lett.* **1968**, *9*, 5907-5908.